APPLE SCAB INFECTION WEATHER IN ENGLAND AND WALES, 1956-60

Contribution from the Conference of Plant Pathologists, National Agricultural Advisory Service, compiled by T. F. Preece, of the Plant Pathology Laboratory, Harpenden, in collaboration with L. P. Smith, of the Meteorological Office, Harrow.

DURING the investigation of ascospore infections in apple orchards at Wisbech, which began in 1953 (Hirst, Storey, Ward and Wilcox, 1955) it soon became evident that means were needed to record the occurrence and duration of periods of leaf wetness. A "dew balance," recording the weight of water on a detached apple shoot, devised by Hirst (1954) was used in an orchard at Wisbech, in conjunction with a rain-gauge and thermograph in 1955. This research instrument proved of such value that the Instrument Development branch of the Meteorological Office at Harrow, in collaboration with Dr. Hirst at Rothamsted, designed a similar and more robust "surface-wetness recorder." This worked on the same principle as the dew balance, but with an artificial wettable element instead of a living apple shoot (Hirst, 1957).

In 1956, at the suggestion of Dr. I. F. Storey, an investigation was begun by the Plant Pathologists of the National Agricultural Advisory Service, in collaboration with the Plant Pathology Laboratory at Harpenden and the Agricultural Branch of the Meteorological Office. The objects of this work were to obtain records of the incidence of scab weather and of subsequent infections in all parts of the country, and to compare these findings, if possible, with the weather conditions already being recorded at standard weather stations. The leaves of unsprayed trees were closely examined in several districts in order to observe the dates on which new lesions became visible, and these dates were considered in relation to records of Mills periods (Mills and La Plante, 1954) and also to other meteorological criteria which appeared relevant as the investigation proceeded.

At first the Mills periods were obtained by direct visual observation of the periods over which the leaves were wet, with readings of maximum and minimum thermometers for the mean temperatures over these periods. As more surface-wetness recorders became available these were tried out and used at several centres, together with thermographs for the temperature recording. At the end of the first year's work it was seen that the pattern of wet days (as defined on page 44) bore some relation to that of Mills periods in different parts of the country; and in 1957 Smith showed that the duration of 90 per cent relative humidity following rain agreed closely with the duration of leaf wetness as obtained by surface-wetness recorders (Smith, 1961; Hearn, 1961).

The present paper is a review of the work over five years, with particular reference to the correspondence between "Smith periods" (as defined on page 44) and "Mills periods" in the various regions; and the relation of both these periods to the first scab lesions seen in each year. The period from bud burst to the end of May only is considered. The importance of the rainfall over this period is shown on pages 52-3 in this issue (Preece, 1961).

First infections are possible as soon as the buds burst at the end of March or early April, and ascospores are being discharged from over-wintered leaves in both May and April. Conidia from twigs with wood scab may also be

available in these two months, and the young growth of foliage and fruit is highly susceptible to primary infection at this time—from bud burst, through pink bud, blossoming and petal fall to the young fruitlet stage.

EXPERIENCE IN 1956

In 1956, observations were made on unsprayed trees at 16 centres, as shown in Fig. 1. In this year a wetness recorder was used at only one centre (Wisbech); at all the other centres the Mills periods were determined by direct visual observation of the time the leaves were wet. An important finding of the year's work was that, in general, growers had no great difficulty in recording Mills periods by this means. Many trees were past bud burst when Mills periods were reported during the period April 9–13 from all recording sites; and widespread Mills periods were reported again on May 9–10.

In 1960 Smith periods were plotted at Harrow for 1956 and subsequent years from the observation books of a number of weather stations selected to represent the several regions. The Smith period chart for the spring of 1956 included in Fig. 2 shows a pattern very similar to that of the recorded Mills periods and it also shows that Mills periods were probably of widespread occurence about March 20, before leaf wetness recording began—but they would have been before bud burst, and therefore before there was any foliage exposed to infection. First scab lesions were seen near 10 of the recording sites during the period May 4–13. Eight of these records were before the widespread Mills periods of May 9–10, and in general the first lesions were seen a

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DEFINITIONS

Throughout this paper the terms "Mills Period," "Smith Period," "Wet Days" and "Rainfall Deviation" are used with meaning as follows:

MILLS PERIOD. A period of leaf-wetness satisfying the requirements for light infection by ascopores, as set out in Table I of Mills and La Plante (1954), e.g.:

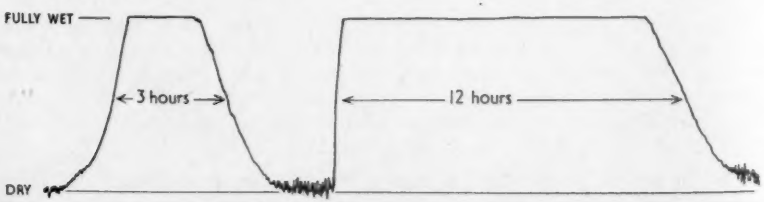
Mean temperature over period °F ...	42	45	50	55	59	60
Hours of leaf-wetness	30	20	14	11	10	9½

SMITH PERIOD. As for Mills Period, but with hours of 90 per cent relative humidity or more, following rain, substituted for hours of leaf wetness in the above table.

WET DAYS. Days on which the rainfall was 0·1 inches or more irrespective of temperature at any particular site.

RAINFALL DEVIATION. Deviation from the monthly long term average rainfall in inches at Cambridge Botanic Garden.

In the estimation of hours of leaf wetness from the daily charts of the surface-wetness recorders, the duration was arbitrarily measured between points half way up the charts, ignoring minor fluctuations of the trace and breaks of not more than four hours, as in the following example:



Length of wet period 15 hours

month after the nationally recorded Mills periods following bud burst, on April 9–13. At the other centres, however, first infections were not seen until the end of May or early June.

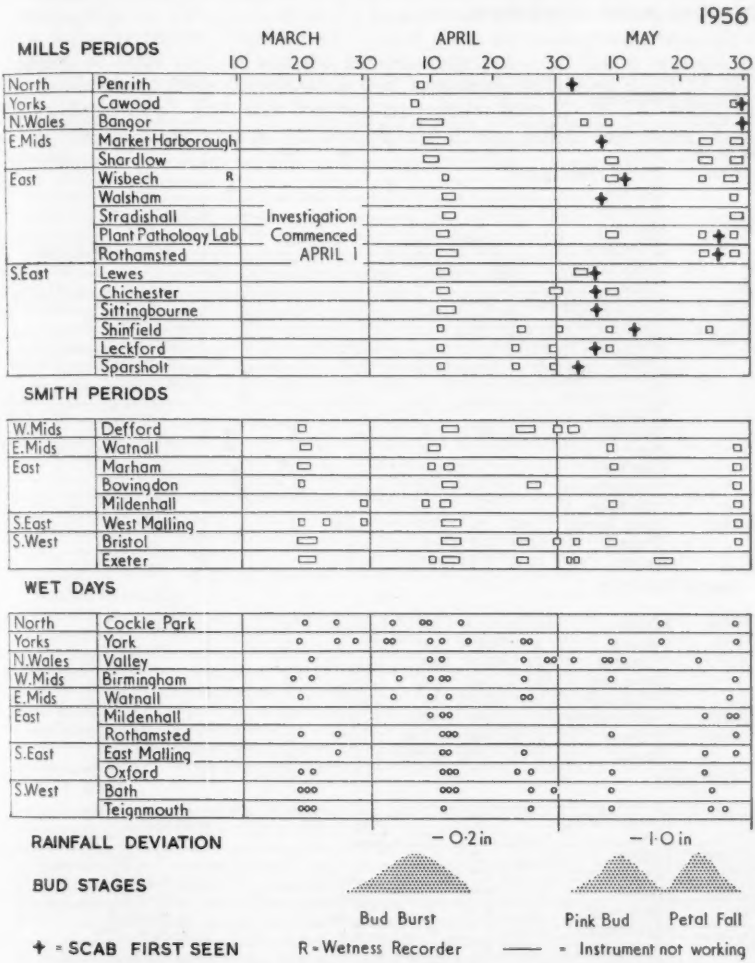


Fig. 1. Weather data, bud stages and first scab infections, 1956.

EXPERIENCE IN 1957

In 1957, wetness recorders were used at five of the 24 centres and visual assessment of leaf wetness at the others. Fig. 2 shows that in general bud burst—in mid-March—was three weeks earlier than in 1956. Mills periods were recorded at all but two of the sites about March 25–28 when the trees were at the early green cluster stage. First scab lesions were seen throughout

the country during the last week in April; and at two centres in the South West and at Bangor in North Wales before this. There was again a good correspondence between the pattern of Mills periods and Smith periods after bud burst, but there were more Smith periods than recorded Mills periods during the actual period of bud burst.

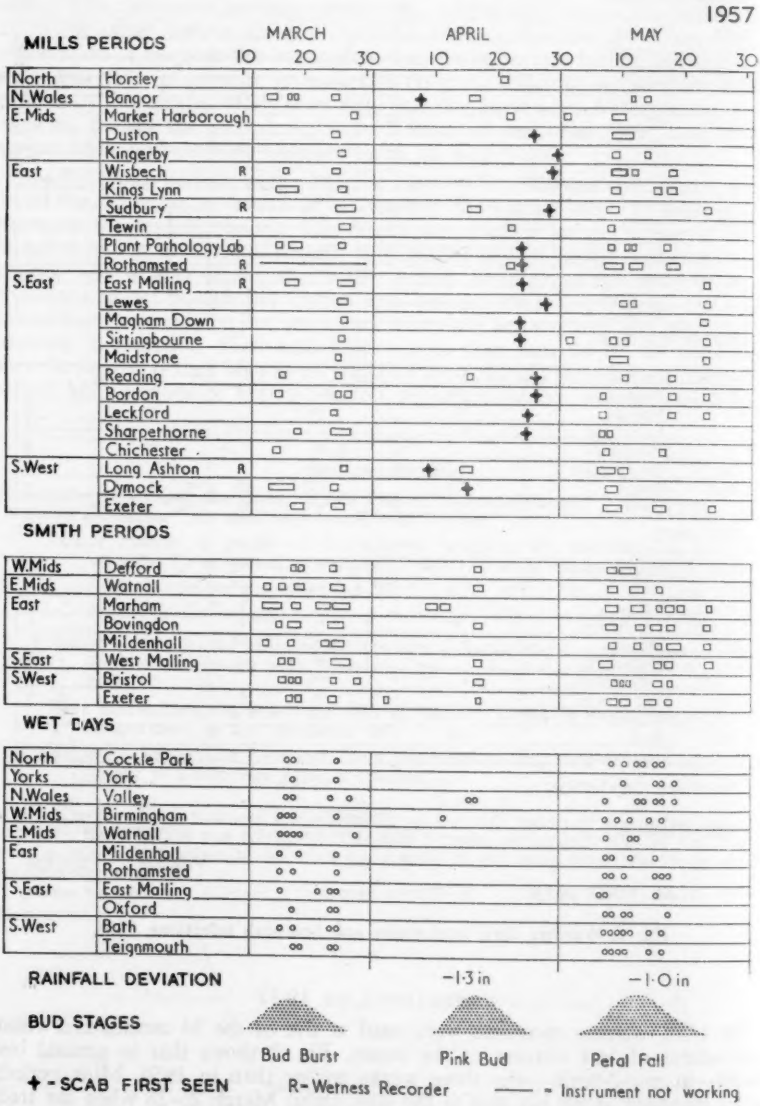


Fig. 2. Weather data, bud stages and first scab infections, 1957.

EXPERIENCE IN 1958

By 1958, surface-wetness recorders based on the prototype built at Harrow had gone into limited commercial production and these instruments (with accompanying thermographs) were used in place of visual observations at 13 centres (Fig. 3). The charts from the instruments were sent weekly to Harpenden for examination. Again there was a good correspondence between the pattern of Mills periods and the subsequently extracted Smith periods. The first widespread Mills periods after bud burst occurred about April 24–28 (at late green cluster). First scab lesions were seen on May 17–26. The widespread Mills and Smith periods at the end of March were before bud burst at most centres.

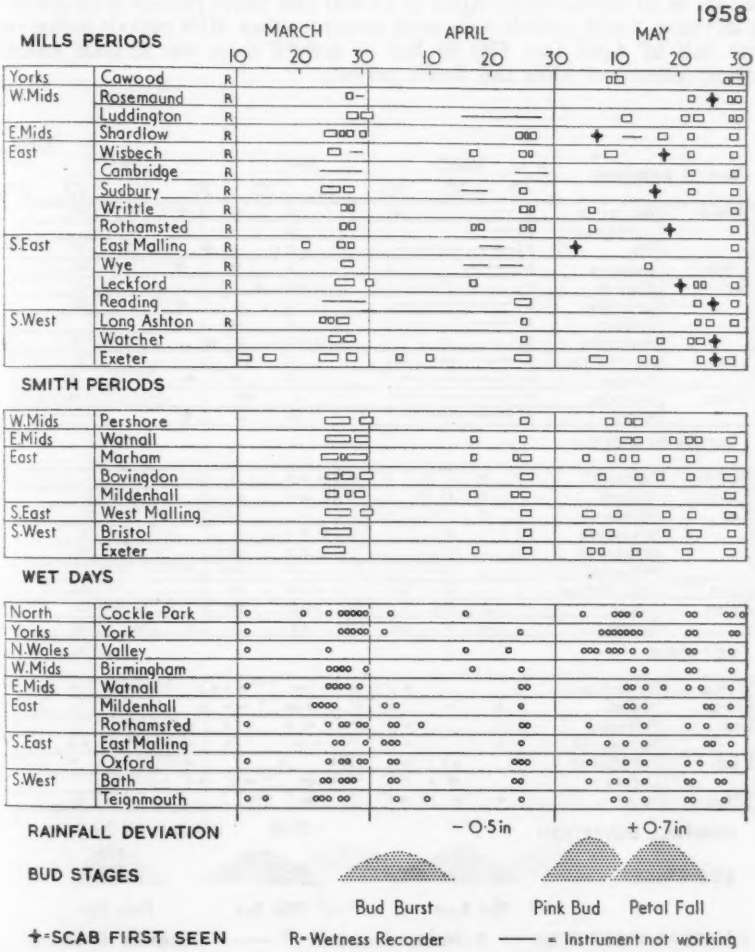


Fig. 3. Weather data, bud stages and first scab infections, 1958.

EXPERIENCE IN 1959

In 1959, wetness recorders were employed at 12 centres (see Fig. 4) under the surveillance of the Regional Plant Pathologists, partly as an aid to advisory work, and partly to provide data for a further year in the present investigation. The Plant Pathologists made their own assessment of Mills periods from the instrument charts, and these were collated at Harpenden. Fig. 4 shows that if Smith periods had been extracted during the spring of 1959, they would have indicated the likelihood of infection periods about March 22, during bud burst, when owing to late delivery of new instruments it had not been possible to have wetness recorders working at all the centres. Scab lesions were seen at most of the centres during the last week in April or first three days in May, again about one month after scab weather following bud burst. There were Mills periods at all centres about April 15–17 and also Smith periods in all districts at this time. Smith periods were more numerous than Mills periods during the first half of April (see Fig. 4) but in general there was a good overall correspondence of Mills and Smith periods.

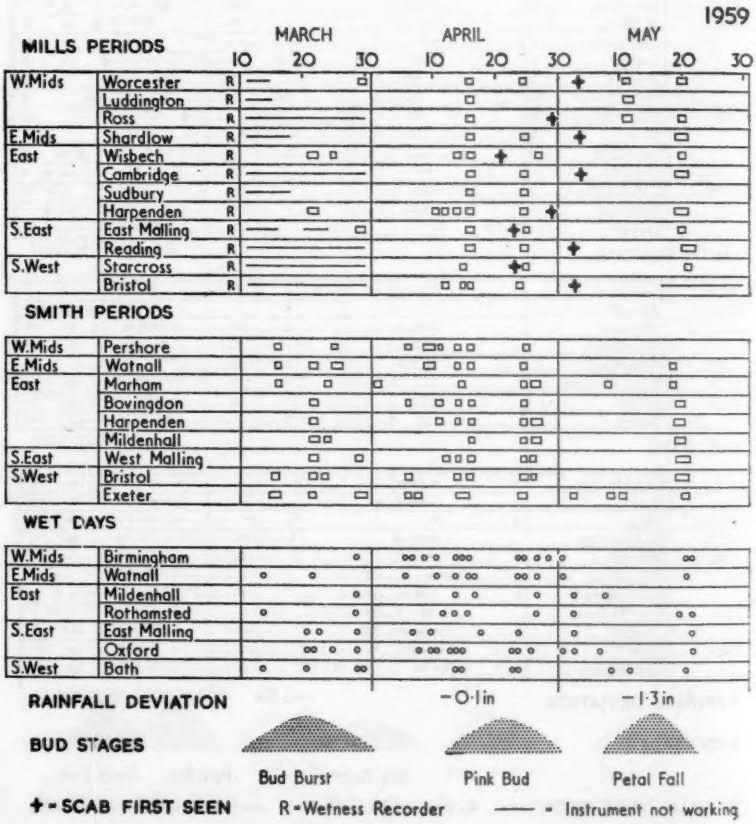


Fig. 4. Weather data, bud stages and first scab infections, 1959.

EXPERIENCE IN 1960

In 1960 an attempt was made to provide Regional Plant Pathologists with Smith period data, as an aid to their advisory work. The extraction of high humidity data is not easy, as reference has to be made to the logs of hourly observations which are compiled at the selected synoptic weather stations; nevertheless by communication over the telephone with observers at these stations, it proved possible for Miss J. Hearn of the Agricultural Unit, Meteorological Office, Harrow, to advise the Plant Pathology Laboratory at Harpenden of the Smith periods that had occurred at the selected stations within four hours of a request for this information, at any time from mid-March to the end of May. Over this period the Plant Pathology Laboratory maintained close liaison with the Regional Plant Pathologist concerned.

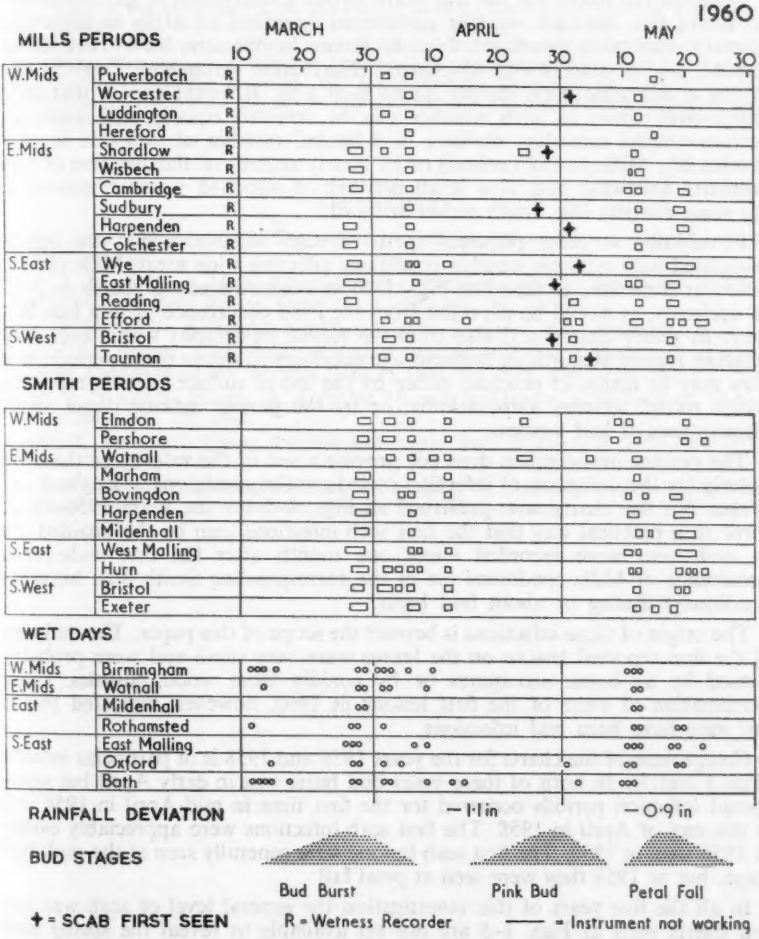


Fig. 5. Weather data, bud stages and first scab infections, 1960.

The chart (Fig. 5) shows the guidance provided by the closely corresponding Mills and Smith periods at and after bud burst in this year. First scab lesions were noted during the period April 26–May 4. There was a brief period on April 12–13 when Smith periods were reported from all regions without corresponding Mills periods, except at Wye. Apart from this there was no widespread infection weather between the end of bud burst and the beginning of petal fall. There was close agreement between the patterns of the Mills and Smith periods during petal fall. Infection conditions were severe at this time, notably in parts of Kent, and many growers reported scab lesions at the fruitlet stage.

DISCUSSION

Study of the charts for the five years of this investigation (Figs. 1–5) leaves no doubt that the scab weather conditions, described by Mills as permitting primary ascospore infection of apple leaves in Western New York State, U.S.A., are of general and widespread distribution throughout England and Wales at definable dates during spring each year. It would appear that these nation-wide times of scab weather can be detected equally well either by surface-wetness recorders working in a limited number of orchards in each district (the Mills period method) or by hourly records of the duration of high humidity following rain at a small network of standard weather stations in the same districts (the Smith period method).

In addition to these principal spells of scab weather, which are usually associated with synoptic weather conditions affecting wide areas, Mills periods do occur from time to time (see Figs. 1–5) in localised areas, orchards or parts of orchards, as would be expected from the local occurrence of rain (see Wet Days in yearly charts) and also from the varied topography within each fruit growing region. Records in orchards are necessary to detect these periods, and they may be made, in practice, either by the use of surface-wetness recorders which record wetness automatically, or by the grower making direct visual observations of leaf wetness.

The present investigation does not provide a test of the validity of the Mills criteria for the definition of infection periods under conditions in England and Wales, but the charts now published as Figs. 1–5 for the years 1956–60 do show in a practical way that the first scab infections seen by the unaided eye in each year were recorded about one month after the first widespread occurrence of Mills conditions (or of the corresponding Smith high humidity conditions) during or about bud burst.

The origin of these infections is beyond the scope of this paper. The majority of the first reported lesions on the leaves were very small and were probably caused by air-borne ascospores or by conidia from wood pustules. Close examination of some of the first lesions in 1960, however, indicated that a few may have been bud infections.

Comparison of the charts for the years 1956 and 1958 is of particular interest (Figs 1 and 3). In both of these years bud burst was in early April but widespread infection periods occurred for the first time in mid-April in 1956 and at the end of April in 1958. The first scab infections were appreciably earlier in 1956 than in 1958. The first scab lesions were generally seen at the pink bud stage, but in 1958 they were seen at petal fall.

In all the five years of this investigation the general level of scab was low and charts such as Figs. 1–5 are not yet available to reveal the spring scab weather pattern in a really bad scab year.

SUMMARY

Investigations carried out at a network of centres in the principal apple growing regions of England over the years 1956–60 have indicated that the occurrence of the main, widespread meteorological conditions favouring infection in the spring can be determined equally well by observation of periods of high humidity after rain at standard weather stations, or by the observation of Mills periods using wetness recorders in representative orchards. The name "Smith periods" has been used to designate the periods defined by the humidity criteria employed.

The Conference of Plant Pathologists thanks all those growers, horticultural officers, research workers and others who have contributed to the work over the five years. Particular thanks are due to Dr. I. F. Storey of the N.A.A.S., Shardlow, and to Dr. J. M. Hirst of the Rothamsted Experimental Station, for their help on working parties in the early stages of the investigation. At the Plant Pathology Laboratory, the first year's work was organised and collated by Mr. E. C. Large: Mr. T. F. Preece assisted with the collation in 1957, and thereafter became responsible for the work. In the Agricultural Unit of the Meteorological Office at Harrow, Miss Jacqueline Hearn devoted much of the winter of 1959 to the extraction of "Smith periods" from past weather records, and in 1960 was responsible for the day to day determination of these periods for test of their operational possibilities.

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SPRING RAINFALL AND APPLE SCAB IN ENGLAND, 1915-60

by T. F. PREECE

Plant Pathology Laboratory, Harpenden, Herts

It was pointed out by Bremer (1924), that from 1897 to 1908 in Germany, the amount of rain during the second half of April or May, according to district, determined the severity of attacks by the apple scab fungus (*Venturia inaequalis* (Cooke) Wint.). He argued from this that the first and most important of the Bordeaux mixture sprays then being used for scab control should be applied immediately after any heavy rain in this pre-blossom period. W. C. Moore (1943), reviewing experience in England over the years 1933–42, recorded that a wet May had by then come to be regarded as the portent of a bad scab year.

During 1960, the original records of scab severity sent to the Plant Pathology Laboratory since 1915 were re-examined and tabulated alongside the deviations from the long-term average of the rainfall during both April and May at Cambridge—which was chosen as the most suitable inland indicator station (Fig. 1).

Scab attacks were severe at Wisbech and in the West Midlands during 1948–51 and in 1953. The April rainfall in these years was above average, and in four of them the May rainfall was also above average. Conversely, during the recent years 1954–60 the April rainfall was below average, as also was the May rainfall in 1956–57 and in 1959–60. These were not scab years, and the picture was similar during 1940–47.

The significance of April rainfall is also indicated by records of wet days. The mean number of days with 0·1 inches of rain or more in April and May at a group of six indicator stations—Birmingham, Watnall, Rothamsted, East Malling, Oxford and Bath—over the years 1950–60, with figures for scab years in heavy type, were as follows:

	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960
April	**6**	**10**	5	**8**	2	3	4	0	4	6	3
May	**6**	**9**	8	**6**	5	12	6	5	6	3	3

Charts showing the pattern of wet days at individual stations for the years 1956–60 are given by Preece and Smith (1961), where their relation to periods of leaf wetness and other meteorological criteria are considered.

From a study of the records over the whole period 1915–60 it would appear that the total rainfall in April usually determines the severity of scab attacks in England; but that many of the years with a wet April also have a wet May.

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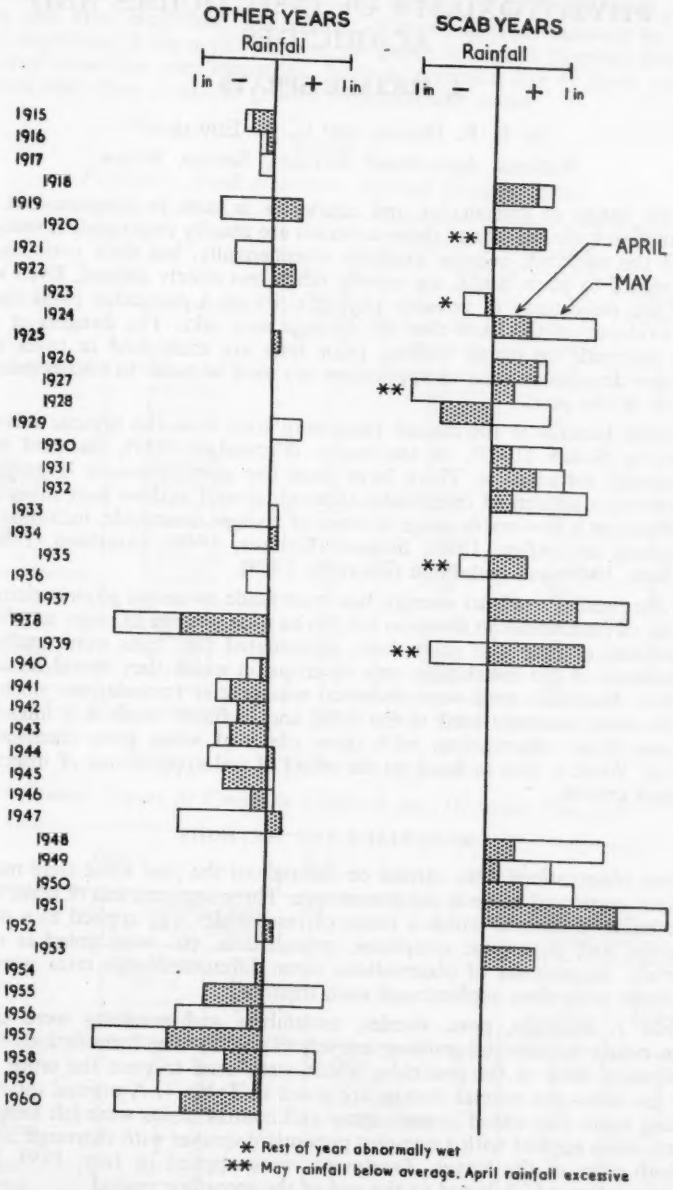

Fig. 1. Incidence of Scab-years in principal English apple growing areas in relation to deviation from average of April and May rainfall at Cambridge, 1915–60.

PHYTOTOXICITY OF INSECTICIDES AND ACARICIDES

I. FOLIAGE SPRAYS

by E. B. DENNIS *and* C. A. EDWARDS *
National Agricultural Advisory Service, Bristol

A WIDE range of insecticides and acaricides is now in common use. The mammalian toxic hazards of these materials are usually thoroughly investigated before the materials become available commercially, but their toxic hazards with regard to plant health are usually much less clearly defined. Even where there are indications of possible phytotoxicity to a particular plant there is little evidence of the form that the damage may take. The dangers of using these materials on plants without prior tests are multiplied in cases where excessive dosages or rates of application are used in order to obtain enhanced control of the pest.

Further hazards to the treated plant may arise from the organic solvent as shown by Stoker (1947), or the wetter (Furmidge, 1959), included in the commercial formulation. There have been few comprehensive investigations on phytotoxic aspects of insecticides although several authors have investigated the effects on a reasonable range of crops of a single insecticide, including DDT (Chapman and Allen, 1948), lindane (Rohwer, 1949), parathion (Pritchard and Beer, 1949) and malathion (Boocock, 1959).

In the present work an attempt has been made to access phytotoxicity of a number of insecticides in common use, on as wide a range of crops as possible. Limitations of time and space have necessitated that tests were confined to applications of the insecticides only to crops on which they would be used in practice. Materials used were technical commercial formulations since these are the ones generally used in the field, and in future work it is intended to compare these observations with those obtained when pure chemicals are applied. Work is also in hand on the effect of soil applications of insecticides on plant growth.

MATERIALS AND METHODS

Since observations were carried on throughout the year some were made on outdoor crops and some in the greenhouse. Three separate sets of observations were made in each of which a range of insecticides was applied to a number of crops, and phytotoxic symptoms, growth data, etc. were noted at regular intervals. In each set of observations three different dosage rates were used and there were three replicates of each treatment.

Trial 1. Parsnips, peas, swedes, cucumbers and tomatoes were sprayed when nearly mature and growing actively (Table 2). The formulations and the application rates of the pesticides which were used to treat the crops at five and ten times the normal dosage are given in Table 1. A normal quantity of wetting agent was added to each spray and control plants were left unsprayed. Sprays were applied with a one-pint pneumatic sprayer with thorough coverage of both sides of the leaves. Treatments were applied in July, 1959, in hot, sunny weather which lasted to the end of the recording period.

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* Now at Rothamsted Experimental Station, Harpenden, Herts

Records were kept of the condition and growth stage of individual plants before and after treatment. The results of spraying were assessed by close visual inspection of the aerial parts of the plants at regular frequent intervals beginning three days after treatment. At each inspection size of plant, number of leaves and other details of growth were carefully noted.

TABLE 1

Pesticides Used and their Normal Dosage Rates

Pesticide	Formulation	*Per cent* active ingredient	Normal Dosage Rate		Used in Trials No.
			per cent active ingredient in spray	Fl. ozs. per 100 galls.	
BHC (gamma) ...	* Miscible	10	0·03	50	1, 2
DDT	Miscible	25	0·10	60	2
DDT	Emulsion	15	0·10	100	2, 3
Aldrin	* Miscible	30	0·30 †	160	2
Dieldrin ...	* Miscible	15	0·05	50	2, 3
Kelthane ...	Emulsion	18½	0·01	8	2
Phenkaptone ...	Emulsion	20	0·02	16	2
Malathion ...	Miscible	50	0·10	30	1, 2
Parathion ...	* Miscible	20	0·04 †	30	2
Diazinon ...	* Emulsion	20	0·02	16	1, 2
Rogor	Emulsion	40	0·04	16	All
Demeton Methyl	Miscible	50	0·04	12	2, 3
TEPP	* Miscible	20	0·02	8	2, 3
Phosdrin ...	Concentrate	99	0·025	4	2
Fenson	* Miscible	20	0·05	40	2
Chlorbenside ...	Miscible	20	0·012	10	2
Fluoracetamide	* Miscible	15	0·02	20	2

* Plus wetter in formulation. † Rates normally used in soil treatments.

TABLE 2

Growth Stages of Crops in Trials II and III when Treated

Trial 2			Trial 3		
Crop	Growth Stage when treated		Crop	Growth Stage when treated	
	Av. height above ground (inches)	No. of leaves		Av. Height above ground (inches)	No. of Leaves
Peas	6	5	Peas	6	4
Beans	12	7	Beans	7	3
Lettuce ...	4	6	Lettuce ...	3½	5
Fodder Beet ...	6	5	Fodder Beet ...	5	5
Strawberries ...	5	9			
Cabbage ...	4	5			
Tomatoes ...	10	7			
Cucumbers ...	27	8			

Trial II. Outdoor crops of peas, beans, lettuce, fodder beet, cabbage and greenhouse crops of tomatoes and cucumbers were grown from seed in pots

of J.I. potting compost No. 1, and artificial feeds were added to ensure normal growth. Pesticides listed in Table 1 were applied to these crops as sprays at once, twice and five times normal dosage rates. Where a range of dosage rates were recommended the highest one was taken as the normal rate. Sprays were applied as in Trial 1 but wetters were not used except where they were already present in the formulation (Table 1). Treatments were applied on September 16 and there were three replicates of each dosage rate used. Growth stages of crops at time of treatments are listed in Table 2.

Trial III. A second crop of peas, beans, lettuce, fodder beet and cabbage was grown in the same way but in this case J. I. compost No. 2 was used.

The plants were transferred to a warm greenhouse eight days after application of the insecticides which was on October 13, 1959, and, in this trial, an amount of wetter was added to all sprays equivalent to the chemical dosage rate. Otherwise treatments were applied as in the previous trials.

The minimum dosages which resulted in damage in any form are summarized in Tables 3 and 4. It should be noted that in the majority of cases the plants recovered from the damage except in the cases of the highest dosages of the more harmful chemicals. There was a wide variety of responses to the various chemicals and these are summarized in the following list.

CHLORINATED HYDROCARBONS

Lindane. This insecticide was generally only slightly phytotoxic, and no damage occurred at dosages below five times normal. Typical phytotoxic symptoms consisted of slight edge chlorosis and scorch, combined with a marked tendency for distortion of the leaves, which curled at the edges, wrinkled and drooped (Plate III, 7). Occasionally these symptoms were succeeded by a speckled scorch. Cucumbers and tomatoes gave anomalous results. Outdoor tomatoes were decidedly sensitive to sprays of five times normal strength with wetter, but greenhouse tomatoes were not damaged at this dosage rate by a spray lacking wetter. Cucumbers were unaffected by the 0·15 per cent spray with wetter when grown outdoors, but were slightly damaged by this spray without wetter in the greenhouse.

DDT Emulsion. This formulation of DDT was rather markedly phytotoxic. At normal dosage rates, damage was severe to cucumbers and slight to tomatoes, beans, cabbage and peas (Plate III, 4) in the greenhouse, but when no wetter was present cabbage was undamaged. Outdoors, strawberries showed severe chlorosis of young leaves at normal dosage rates.

Damage generally took the form of scorch, general chlorosis, distortion of the leaves and growing point. The most characteristic feature at higher dosage rates was almost complete cessation of growth (Plate II, 6). Tomatoes showed mottled chlorosis of the upper leaves: peas and fodder beet were scorched. Cucumbers were killed at normal dosages (Plate II, 9).

DDT miscible. DDT in this formulation was only applied to strawberries, and it resulted in chlorosis of the young leaves as was seen with DDT emulsion, but the symptoms were less marked and no scorch developed. Similar damage occurred at all the dosage rates used.

Aldrin miscible. The rates used for this material were those normally applied as soil treatments. At this rate aldrin was markedly phytotoxic particularly to beans and tomatoes. Where symptoms were slight they took the form of marginal leaf scorch and bronzing, recurved leaf edges, slightly twisted growing tips, chlorosis and wilting. At higher dosages plants were stunted, with the growing points severely scorched and in some cases completely killed (Plate III, 8).

Dieldrin. Only moderate phytotoxicity was attributable to dieldrin. Peas and cabbage (Plate III, 3) were the most sensitive crops but there was some damage to beans at normal rates, and to lettuce at the highest rates used. More damage resulted from sprays with wetters added. At lower dosage rates the early symptoms consisted of slight leaf edge scorch and chlorosis with the leaves slightly puckered and twisted. Severe scorch, wrinkled leaves, dwarfing and general chlorosis were the more typical of the high dosages. Typical damage to fodder beet consisted of small scorch ringspots.

Kelthane. Kelthane was tested only on strawberries and cucumbers. Damage to the latter crop was severe, and took the form of chlorosis, scorch and considerable twisting of the leaves, particularly at the higher dosage rates (Plate II, 8).

ORGANOPHOSPHORUS MATERIALS

Phenkaptone. This material was tested only on tomatoes and damage in the form of slight chlorosis and tip scorch was confined to the highest dosage rate.

Malathion. Only relatively mild damage was caused and only strawberry plants were sensitive to malathion at a low dosage rate. At five times the normal rate of application, slight damage occurred to all the crops tested. The symptoms were generally unspectacular and included slight chlorosis and leaf edge scorch. More severe symptoms consisted of extreme leaf edge scorch and chlorosis and some twisting of the growing points. These symptoms were most marked in outdoor cucumbers, which were killed by a 0·5 per cent spray.

Parathion. The dosages used were higher than intended, but very little damage was caused to the crops tested even at this dosage. Cucumbers (Plate II, 10) and strawberries were slightly affected by a 0·04 per cent spray, but tomatoes showed no damage until a 0·20 per cent spray was applied. General symptoms were some scorch, leaf curl and distortion. Strawberry plants showed a distinct chlorosis of the young leaves.

Diazinon. At normal and twice normal dosages diazinon did not cause damage to any of the crops. At 0·10 per cent damage occurred to all the crops tested and in some cases the damage was severe. Where damage was slight, as with tomatoes, beans, peas, swedes, parsnips and outdoor cucumbers, the symptoms generally consisted of tip scorch and edge chlorosis and scorch combined with a tendency for the leaves and branches to droop (Plate III, 5). Severe damage which occurred to most crops with 0·20 per cent sprays consisted of bad scorch, chlorosis and distortion, leading to the eventual death of the growing point (Plate III, 1).

Rogor. Considerable damage was caused to some crops. Beans and cucumbers were affected by the normal spray, peas by a twice normal spray, but most crops showed more or less severe symptoms when a five times normal spray was applied. Marked edge chlorosis (Plate III, 2) and edge and tip scorch were the most typical symptoms of damage, but on some crops mild chlorosis and scorch speckling were also noted and there was some leaf curl (Plate II, 3). On fodder beet small circular necrotic lesions occurred similar to those produced by other pesticides on this crop.

Demeton-methyl. This appeared to be the safest material of all those tested. There was a slight tendency to cause general chlorosis at the higher dosage rates. Otherwise the only damage recorded was a very slight scorch on cabbage and peas at the highest rate.

TEPP. Indoor cucumbers, tomatoes and lettuce (Plate II, 4) were damaged even at normal dosage rates, and the damage to tomatoes was severe (Plate III, 6). Only slight damage to peas, fodder beet and beans occurred with the 0·04 per cent sprays, but the 0·10 per cent sprays produced severe symptoms on all crops tested other than cabbage (Plate II, 1). The slight symptoms noted included mild scorch, leaf crinkle, edge curl and scorch. When severe, damage consisted of serious scorch and distortion and finally death of the growing point.

OTHER MATERIALS

Fenson. Both indoor cucumbers and tomatoes were slightly damaged at normal dosage rates. Higher dosages caused more severe damage to cucumbers (Plate II, 7) but not tomatoes. The damage to tomatoes consisted of tip scorch, mild chlorosis and leaf curl. The severe damage to cucumbers took the form of bad scorch, marked leaf curl, twisted main stem and death of growing point.

Chlorbenside. Cucumbers treated at the normal rate were unaffected, but at twice and five times this dosage a slight edge scorch and chlorosis developed.

Fluoracetamide. No damage at all occurred as a result of treatments to the only two crops tested, fodder beet and cabbage.

TABLE 3

Minimum Dosages of Pesticides to cause Phytotoxicity

Pesticide (No wetter)	Normal Dosage Rate *per cent* a.i.	Crop	Minimum Dosage *per cent* a.i. to cause Phytotoxicity
Aldrin	0·075	Tomato	1·50
		Lettuce	0·60
		Beans	0·30
DDT Miscible ...	0·10	Strawberry	0·10
Kelthane	0·01	Cucumber	0·01
		Strawberry	N5
Phenkaptone	0·02	Tomato	0·10
Phosdrin	0·025	Strawberry	0·10
Fenson	0·05	Cucumber	0·05
		Tomato	0·05
Chlorbenside	0·012	Cucumber	0·02
Fluoracetamide ...	0·02	Fodder Beet	N5
		Cabbage	N5
Parathion	0·015	Cucumber	0·04
		Tomato	0·20
		Strawberry	0·04

N5 = No damage at five times normal dosage rates. All indoor cucumbers and tomatoes.

DISCUSSION

In these investigations technical commercially produced grades of chemical were used. There are sound arguments in favour of using pure chemicals in place of these, but it was felt that the first importance was to indicate the dangers of using particular chemicals in the formulations most commonly used in practice. This approach does however, raise the objection that damage observed in the experiments may be due to impurities in the chemicals, the carrier or to the presence or absence of a wetter. It is known from previous work that any of these factors may cause damage to plants, as in Furmidge's work which has investigated phytotoxic effects of wetters in fruit tree sprays.

In the present work only one formulation of each chemical was used (except in the use of DDT on strawberries), because of limitations of time and facilities for growing crops. It is intended that this work should be continued to further investigate the effects of the pure chemical, formulation material and wetting agents.

TABLE 4

Minimum Dosages of Pesticides to cause Phytotoxicity

		BHC-Lindane	DDT Emulsion	Dieldrin	Malathion	Diazinon	Rogor	Demeton-Methyl	TEPP
Normal dosage *per cent* a.i.		0·03	0·10	0·05	0·1	0·02	0·04	0·04	0·02
Wetter									
Cucumber									
(Indoor)	−	0·15	0·10		0·50		0·04	N5	0·02
(Outdoor)	+	0·30			0·50	0·20	0·20		
Tomato									
(Indoor)	−	N5	0·10		0·50	0·10	0·20	N5	0·20
(Outdoor)	+	0·15			0·50	0·10	0·08		
Lettuce	+		0·20	0·25			0·20	N5	0·02
	−	N5			N5		0·20	N5	
Beans	+	0·15	0·10	0·10			0·04	N5	0·04
	−	0·15			0·50	0·10	0·08	N5	
Fodder Beet	+		0·20	0·25			0·20	N5	0·04
	−	0·15	0·20					N5	
Cabbage	+		0·10	0·10			0·20	N5	N5
	−		0·20	0·25	N5			0·20	
Peas	+		0·10	0·05			0·08	N5	0·04
	−	0·15	0·20		0·50	0·10	0·08	0·20	
Strawberry	−		0·10		0·20		0·20	N5	
Swedes	+	0·15			0·50	0·10	0·20		
Parsnips		N10			0·50	0·10	0·40		

N5 = No damage at 5 times normal dosage. N10 = No damage at 10 times normal dosage.

Differences, in fact, were obtained where the same crops were sprayed with chemicals with and without a wetter. In some cases the addition of a wetter increased the phytotoxic effects of the spray, as for instance in the treatment of cabbage with DDT and dieldrin. A similar effect was noted with DDT on peas and Rogor on beans. In one treatment however the addition of a wetter resulted in less damage than with the spray alone. This was demeton-methyl which caused slight damage to cabbage and peas without a wetter but when a wetter was added no damage occurred.

Of the more widely used commercial materials tested in these trials those which caused most damage were DDT, aldrin, dieldrin, Rogor, TEPP and parathion. Nevertheless in few cases was damage to the crop so extreme that there was no recovery, although in a number of cases a severe check to the crop resulted sufficient to cause serious losses in commercial practice. In the majority of cases damage was confined to the older foliage, except where damage was severe and the crop particularly sensitive to the chemical, e.g. cucumbers treated with DDT, the new growth was also damaged. Chemicals with a more restricted use which caused damage in some tests were fenson, chlorbenside and Kelthane. The remainder, at least in the formulations tested, were comparatively safe for use even at excessive dosage rates.

It had been hoped that where damage occurred due to a particular chemical it might be so clearly defined that it would be possible to diagnose damage in the field. In some cases it may be possible but in a large number of cases the symptoms to a particular crop caused by a wide range of chemicals were extremely similar, and took the form of early chlorosis and scorch, followed by leaf distortion and general dwarfing. In general the organophosphorus materials tended to cause edge chlorosis and scorch; this was especially true of Rogor but was also seen with malathion and diazinon. The chlorinated hydrocarbons, by comparison, tended to produce distortion, leaf curl and leaf droop but there are many exceptions to these generalizations. At very high dosages most materials severely reduced growth and severe necrosis was a common feature, with severely dwarfed plants in extreme cases.

It was also evident that there was a marked tendency for a particular crop to have a specific reaction to treatment with a number of chemicals. The leaves of beans readily distorted, necrotic spots developed on fodder beet, and young leaves of strawberry became chlorotic. Leaves of tomato plants commonly curled while cucumber leaves were scorched, wilted and died and lettuce leaves yellowed and wilted.

As far as possible all plants were growing actively when treated but it was quite obvious that many factors could affect the susceptibility of any crop to the phytotoxic effects of a particular chemical. Such factors as formulation, growth stage and vigour of growth of the crop and weather conditions could all strongly modify the results. A great deal of further investigational work would appear to be necessary before the interdependence of these operative factors, which govern the appearance of phytotoxic effects, can be more clearly understood.

SUMMARY

Commonly used pesticides were sprayed on the foliage of a range of outdoor and indoor crops for which they would normally be recommended. Once, twice and five times normal dosage rates of chemical were applied, both with an equivalent amount of wetter and without one. Records were kept of the development, description and location of phytotoxic symptoms. Comparatively safe and harmful materials were revealed. Slight and severe phytotoxic symptoms are described for each pesticide: some chemicals caused similar symptoms to appear in a number of crops: frequently crops produced similar symptoms when damaged by different materials.

Our thanks are due to Mr. I. Mathers and his staff who provided and tended the plants for experimentation.

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LUCERNE MOSAIC VIRUS IN GREAT BRITAIN

by A. J. GIBBS *and* T. W. TINSLEY*

Rothamsted Experimental Station, Harpenden, Herts

LUCERNE mosaic virus (LMV) has a wide host range (Price, 1940). It was first found and described in North America (Weimer, 1931), where strains of the virus are found in potato (Porter, 1931), and pepper (Berkeley, 1947) as well as many legumes. It has also been found in Bulgaria (Kovachevski, 1942), Germany (Köhler and Klinkowski, 1954), the Netherlands (Oswald, Rozendaal and van der Want, 1955), Italy (Quantz, 1956) and New Zealand (Fry, 1952). In the British Isles the virus has so far been reported only once—in Majestic and Ulster Premier potatoes growing in County Antrim, Northern Ireland (Richardson and Tinsley, 1956). It is widespread, however, in crops of lucerne, white clover and red clover. The purpose of the present paper is to record this, and to describe the symptoms caused by one of the commonest strains in naturally and artificially infected plants.

We have found differences in the symptoms caused in tobacco by different isolates from lucerne, but when tested by a gel diffusion technique (Mansi, 1958) all isolates proved to be closely related serologically to one another, to an American isolate of LMV, and to a strain of LMV isolated from potato. Isolates may be divided into two groups, depending on whether or not they cause systemic symptoms in French bean (*Phaseolus vulgaris* L. 'Prince') most do not, and of 537 isolates made in 1959 from naturally infected lucerne plants in commercial crops from all parts of England, Scotland and Wales, 528 produced only necrotic local lesions in the primary leaves of French bean plants, whereas nine isolates gave systemic infection in addition to necrotic or chlorotic lesions.

SYMPTOMS ON NATURALLY INFECTED PLANTS

Lucerne. (Medicago sativa L. 'Du Puits'). Most infected plants were stunted, their leaves faintly mottled and striped, and sometimes distorted (Plate I, 1). Symptoms were most severe in the spring, and in hot weather became so slight that infected plants appeared normal.

White clover (Trifolium repens L.) and *Red clover (Trifolium pratense* L.) Symptoms similar to those in lucerne, but less severe.

Lupin (Russell hybrids). Infected plants were stunted and their leaves showed a chlorotic mottle.

SYMPTOMS IN ARTIFICIALLY INFECTED PLANTS

The isolate used in these studies was obtained in 1955 from lucerne growing on Rothamsted farm, and it produced only necrotic lesions in French bean.

Tobacco. (Nicotiana tabacum L. 'White Burley'). Chlorotic and necrotic lesions developed on the inoculated leaves four to eight days after inoculation. Systemically infected leaves showed similar symptoms a few days later. Twenty to thirty days after inoculation the plants began to produce leaves with few or no symptoms, although virus could still be isolated from them. (Plate I, 2). Symptom expression depended on temperature. At temperatures above 24°C symptoms developed quickly but were mild, whereas at lower temperatures symptoms developed more slowly but were more severe.

Nicotiana glutinosa L. Symptoms as in tobacco but usually more severe.

* Now at West African Cocoa Research Institute, Moor Plantation, Ibadan, Nigeria.

French bean. (Phaseolus vulgaris L. 'Prince'). Chocolate brown necrotic lesions developed on inoculated leaves two to four days after inoculation. The virus could rarely be transmitted by inoculation of sap from such leaves to healthy French beans, unless the leaves were ground with a buffer solution of pH 7·0 or higher which also contained a reducing agent such as ascorbic acid.

Chenopodium amaranticolor (Coste and Reyn). Faint partially necrotic lesions developed on inoculated leaves four to eight days after inoculation. Systemically infected young leaves were distorted and showed characteristic necrotic and chlorotic flecks.

Broad bean. (Vicia faba L. 'Seville Longpod'). Large spreading black lesions developed on inoculated leaves six to twelve days after inoculation. Black streaks appeared in the stem above and below the inoculated leaves, and the apex then turned black and died. Infected plants usually died 3–5 weeks after infection.

Pea (Pisum sativum L. 'Alderman') and *Sweet-pea (Lathyrus odoratus* L.) Small brown necrotic lessions developed on the inoculated leaves, which then turned grey and wilted six to twelve days after inoculation; the stem apex later also turned grey, and the whole plant wilted and died within a few days.

PROPERTIES AND TRANSMISSION OF LMV

The virus, in sap from infected tobacco plants, became inactivated in 4 to 20 days at 18°C, whereas it was still infective after 100 days at −10°C. It was inactivated after heating for 10 min. at 65°C, but not at 60°C. The virus was readily transmitted to some hosts by rubbing them with sap from infected tobacco or lucerne. Lucerne was rarely infected by this method, but plants growing in the field, or under glass when kept in darkness at 32–36°C for 24 hours before inoculation, sometimes became infected. The virus was transmitted by *Acyrthosiphon pisum* (Harris) when this aphid was starved for one hour, then allowed to feed for five minutes on a diseased plant and subsequently caged on a healthy plant for 24 hours. In this way LMV was readily transmitted to white clover, broad bean, lucerne, pea, and White Burley tobacco.

We are indebted to J. B. Bancroft, Purdue University, Indiana, U.S.A., who supplied the American isolate of LMV. The photographs were taken by F. Cowland, A.R.P.S.

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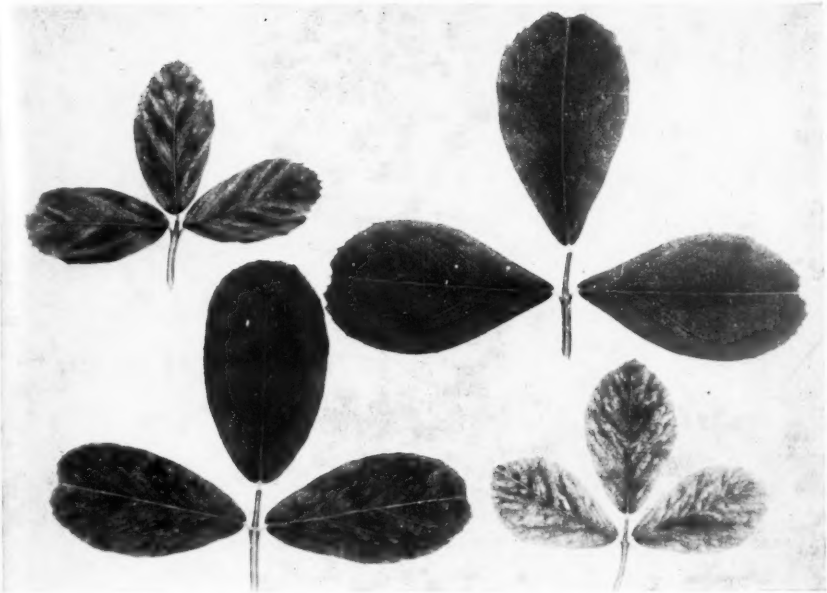


1. Symptoms on Lucerne ('Du Puits'). *Top right:* healthy. *Other three leaves:* infected.

Photos: Rothamsted Experimental Station

2. Symptoms on tobacco. *Left:* inoculated leaf, 10 days after inoculation. *Centre:* systemically infected leaf, 14 days after inoculation. *Right:* leaf from recovered plant, 5 weeks after inoculation.

1. Bean: TEPP 0·1 *per cent* + wetter.

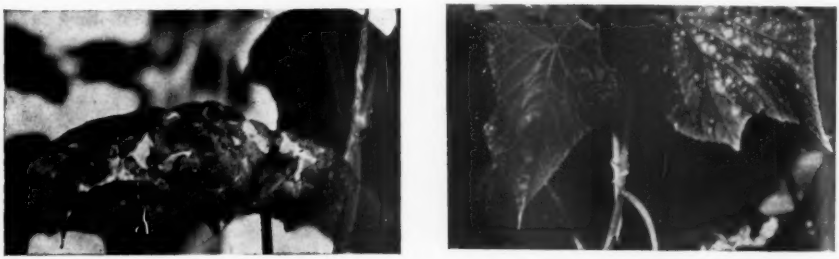
2. Bean: Unsprayed.

3. Bean: Rogor 0·2 *per cent* + wetter.

4. Lettuce: TEPP 0·1 *per cent* + wetter.

5. Lettuce: Unsprayed.

6. Lettuce: DDT 0·5 *per cent.*



7. Cucumber: Fenson 0·1 *per cent.*

8. Cucumber: Kelthane 0·05 *per cent.*

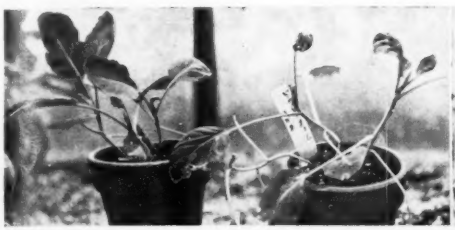
9. Cucumber: DDT 0·5 *per cent.*

10. Cucumber: Parathion 0·2 *per cent.*

PLATE II

1. Swede: Diazinon 0·2 *per cent.*

2. Pea: Rogor 0·2 *per cent* + wetter.



3. Cabbage: Dieldrin 0·25 *per cent.*

4. Pea: DDT 0·5 *per cent.*

5. Tomato: Diazinon 0·1 *per cent.*

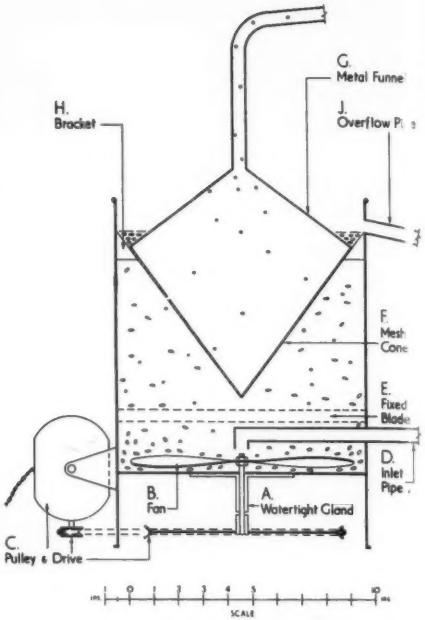
6. Tomato: TEPP 0·1 *per cent.*

7. Tomato: Lindane 0·15 *per cent.*

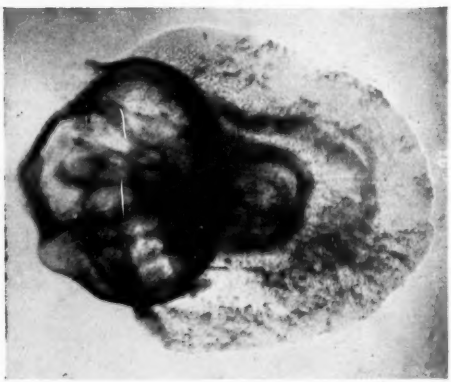
8. Tomato: Aldrin 0·2 *per cent.*

PLATE III

1. Sample of embryos undergoing examination.



2. Construction of "Tea Urn" embryo extractor.



3. Infected barley embryo, showing smut mycelium in scutellum × 20.

4. Grooved examination slide.

Photos: Scientific Services, East Craigs

EXTRACTING BARLEY EMBRYOS FOR LOOSE SMUT EXAMINATION

by W. M. R. LAIDLAW

Plant Pathology Laboratory, Department of Agriculture and Fisheries for Scotland, East Craigs, Edinburgh

A "WHOLE-EMBRYO" method of preparation of barley seed for examination for loose smut, *Ustilago nuda* (Jens.) Rostr., was devised by Russell and Popp (*Sci. Agric.* 1951, **31**, 559–65) in Saskatchewan. In this method the chaff was separated from the embryos by using a set of sieves. In our Laboratory the process had to be speeded up in order to carry out an official certification scheme for barley seed—which involved the examination of a relatively large number of embryos, the limit of tolerance being set at 0·2 per cent. This paper describes two modifications of the Canadian process which have so far proved to be satisfactory.

EXTRACTION WITHOUT DEHULLING

After soaking of the grain in cold* 5 per cent sodium hydroxide overnight the embryos are removed by agitation in hot water in the apparatus shown in Plate IV, 2, which was made originally from a copper tea urn. Hot water is run in through the inlet pipe D, which is arranged so that the flow is directed towards the bottom of the apparatus. The main agitation is effected by the fan blade B, 9½ in diam., the spindle of which passes through the water-tight gland A, and is pulley driven at 1 revolution per second by a 12 volt motor C. The fixed blade E helps to break up the swirl and ensure that the hot water is continuously circulated through the sample. As the embryos become detached from the endosperm they are drawn through the mesh cone F, and siphoned off through the metal funnel G. The funnel-cone assembly is removable and held in place by the fixed brackets H. The embryos are run into a set of graded wire-mesh sieves arranged in descending order of size (6, 8, 10 and 14 meshes to the inch). The overflow pipe J maintains the water level and can be used to remove any floating embryos. A small amount of chaff usually finds its way through the mesh cone and is caught in one of the sieves; also some of the embryos float to the surface and to collect these the hot water input is adjusted so that a slight, steady overflow from J carries them into the sieves along with all the floating chaff.

In practice about 150 g of barley (i.e., about 2,200 seeds yielding 2,000 embryos) is placed in 760 ml of 5 per cent sodium hydoxide solution overnight. It is then transferred to the extractor which is run for 10–20 minutes, the temperature of the water being from 40–60°C. The extraction time can be reduced by raising the temperature of the water, but it must not rise above 65°C or the scutellum will be damaged.

Before removing the embryos from the bottom sieve the set is washed down with water to carry to the bottom any embryos caught in intermediate sieves. The sample is then dehydrated in industrial alcohol and any chaff or endo-

* A method of separation by boiling in 5 per cent sodium hydroxide was described in Denmark by Pederson (*Proc. int. Seed Test. Ass., 1956*, **21**, 112-28). When this method was used on barley seed grown in Scotland the embryos disintegrated, possibly owing to differences in harvesting conditions.

sperm is removed by adding lactophenol solution to the embryos in a filter funnel, when the embryos float and the chaff sinks and can be run off. This last process is repeated until all the debris is removed. Finally the embryos are cleared by boiling for a few minutes in a fresh portion of lactophenol. They are then ready for examination. Lactophenol is used as the mounting medium.

EXTRACTION AFTER DEHULLING

In this method the seed is dehulled in sulphuric acid, and the endosperm dissolved in hot water after treatment in sodium hydroxide. For examination the embryos are cleared in lactic acid and stained in trypan blue. A higher proportion of embryos is obtainable by this method, so a smaller sample of seed, 120 g, is sufficient. This is weighed out and placed in a 2-litre beaker containing 250 ml of 50 per cent commercial sulphuric acid. The large basal area of this beaker allows quick dissolution of the endosperm. The seed remains in the acid for 2–3 hours at room temperature, after which the acid is washed off with any free hulls. The sample is then transferred to an M.S.E. "Ato-Mix" 800 container, three-quarters filled with water, and run at half speed for 2–3 minutes. This loosens the remaining hulls which can then readily be removed by washing. To prevent damage to the grain the cutting blades of the "Ato-Mix" are blunted, filed down until the sides are parallel and covered with rubber tubing which is wired on.

The dehulled sample is now replaced in the beaker and covered with 400 ml of 20 per cent sodium hydroxide solution and allowed to stand overnight. The sodium hydroxide causes some swelling of the endosperm and the embryos become slightly shrunken and partially cleared. The sodium hydroxide solution is poured off and the beaker, filled with hot water is placed in a steamer for 1 to 1½ hours and occasionally stirred. The water, containing some dissolved endosperm, is poured off and the beaker refilled with hot water and replaced in the steamer for a further hour, again with occasional stirring.

The mass of dissolved endosperm is now poured through a set of three sieves, arranged in descending order of size (6, 10 and 20 meshes to the inch) and washed with hot water from a rubber tube or spray head. The endosperm is washed away leaving the embryos in the bottom sieve. A certain amount of debris is retained by the 10-mesh sieve but some may be present along with the embryos on the bottom sieve. Occasionally, depending on the sample and on the method of heating, small particles of undissolved endosperm may appear along with the embryos and can be removed by reheating in the steamer with a small amount of water. The embryos are then placed in a filter funnel with 50 per cent lactic acid. Many of the small pieces of membrane can now be removed as they have a tendency to sink and can be run off first, while the embryos float on the surface of the acid and can be run off later. (A polythene wash bottle filled with 50 per cent lactic acid has been found to be very helpful here).

The embryos are then boiled in a fresh portion of lactic acid and again boiled momentarily in 0·1 per cent trypan blue in 50 per cent lactic acid. After excess stain is washed off in lactic acid the embryos are again boiled, this time for a few moments, in fresh lactic acid to remove the stain from the embryo tissue. They are then ready for examination. 50 per cent lactic acid is used as the mounting medium. The staining procedure is greatly facilitated if the embryos are placed in a basket made of fine wire mesh, 20 per inch, and of such size that it will fit inside a 100 ml beaker. The basket can then be readily transferred to the various solutions required for staining.

EXAMINATION OF THE EMBRYOS

Extraction without dehulling, as here described, has the advantage that the whole process can be carried out in two days with a total working time of about 1–1½ hours, but it has the disadvantage that a good supply of hot water must be readily available. The second method, extraction after dehulling with acid, uses very little hot water and requires no special apparatus, but the working time is slightly greater.

The examination of large numbers of embryos extracted by either method is readily carried out by using a transparent plastic slide of the type shown in Plate IV, 4. The slide is machined out of Perspex sheet 0·2 in. thick, and all the grooves are 0·1 in. deep. The small connecting channels allow the mounting medium to level out in all the grooves, and the peripheral channel stops overflow of mounting medium from the slide. The embryos are run into the grooves where they tend to settle in parallel lines; they are then covered either with lactophenol or lactic acid and examined using a stereoscopic binocular microscope with moveable stage as shown in Plate IV, 1. A practised worker using a hand tally counter can examine 1,000 embryos by this method in 30 minutes. The appearance of an infected embryo under the microscope is shown in Plate IV, 3.

I should like to acknowledge the help I have received from my colleagues on the staff of the Scientific Services, Plant Pathology Laboratory, and in particular from Dr. Mary Noble at whose suggestion this article was written.

OVERWINTERING OF *SPHAEROTHECA HUMULI* ON STRAWBERRY PLANTS

by O. S. PERIES

Horticultural Science Laboratories, University of Bristol

THE powdery mildew of strawberries, *Sphaerotheca humuli* (DC.) Burr., sporulates only during the summer, and its method of overwintering in England has not been established. The powdery mildews as a group exhibit a range of methods of perennation, including overwintering on alternate hosts, survival as chlamydospores or ascospores, hibernation as mycelium in buds, dead leaves or living leaves. The possibilities of these various methods of overwintering of powdery mildew on strawberries were investigated on two-year-old plants of the varieties Coronation, Royal Sovereign, and Cambridge Vigour, kept under observation at Cheddar, at Long Ashton, and in the Horticultural Science Laboratories of the University of Bristol.

Cross inoculations showed that none of the powdery mildews on the weeds present could be transferred to strawberry; and no formation of chlamydospores or thickening of hyphal walls was found in the mycelium of *S. humuli* present on dead mildewed strawberry leaves. Mycelium from such dead leaves, and pieces of dead laminae carrying mycelium, were maintained in contact with healthy strawberry leaves but no infection resulted. A large number of dormant strawberry buds from plants that had shown severe mildew in the previous growing season were collected at Cheddar. These were fixed, sectioned and stained with lactophenol-cotton blue; no mycelium was found

in any of the buds examined. Gilles (1961) recorded the production of perithecia of *S. humuli* on strawberries in France, but reported that the ascospores were not viable. Rashid Kahn (1960) noted an isolated occurrence of perithecia on strawberry runners growing under glass at Long Ashton, but there are no further records of the ascus stage in England.

Gilles (1961) suggested that *S. humuli* on strawberry overwinters as mycelium on old leaves. He found such mycelium, but does not make it clear whether his old leaves were dead or alive; and as he did not obtain sporulation on the mycelium he was unable to identify it with certainty. At Cheddar, overwintering green leaves were collected weekly from October 1959 to May 1960. Hyphae were found on a few of the leaves in each collection. Plants brought in from Cheddar in the early spring and placed under polythene covers in a heated greenhouse produced conidia of *S. humuli*. Hyphae on tagged overwintering leaves, collected in late May 1960 also produced conidia when the leaves were detached and incubated for three days at 20–22°C.

Sporulation of the overwintering hyphae under field conditions was first seen on May 30, 1960. When the overwintering leaves bearing sporing mycelium were decolorized and stained the mycelium was found to be of the same type as that observed on strawberry leaves throughout the winter. It was concluded therefore that in the Cheddar area the source of powdery mildew inoculum for young strawberry leaves in the spring was the conidial growth on mycelium that had remained alive on the overwintering green leaves.

The ability of *S. humuli* to survive in the field through the winter suggests that this fungus is resistant to the effects of low temperature. In laboratory experiments it was found that a small percentage of the conidia germinated at 2°C, and that conidia sown on strawberry leaves remained viable for over 5 weeks when kept in a saturated atmosphere at 0°C. Germ-tubes of the fungus continued to grow slowly at 0°C., and resumed rapid growth when the leaves were transferred to 20°C. The mycelium remained viable for more than 3 weeks on detached leaves kept in a moist atmosphere at 0°C., and sporulated when the leaves were incubated in Petri dishes at 20°C. Although the conidia can survive for a number of days at 0°C., it seems unlikely that they play any part in the perennation of the fungus; a few germinate in a few hours at temperatures above 2°C., and temperatures higher than this are often recorded during the winter in the Cheddar area.

SUMMARY

In detailed observations of two-year-old strawberry plants growing at Cheddar, Somerset, in the winter of 1959-60, it was found that mycelium of *Sphaerotheca humuli* survived on the green overwintering leaves and produced conidia in the spring which caused infection of the young leaves. The mildew was not found to overwinter in any other way.

I thank Dr. T. E. T. Bond of the Horticultural Science Laboratories, University of Bristol, and Mr. R. W. Marsh of Long Ashton Research Station, under whose supervision this work was carried out.

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ON THE DISTRIBUTION AND INFECTIVITY WITH ARABIS MOSAIC VIRUS OF A DAGGER NEMATODE

by R. S. PITCHER *and* ASHARFI JHA*
East Malling Research Station

Now that the dagger nematode *Xiphinema diversicaudatum* (Micol., 1923 and 1927) Thorne, 1939 has been shown to be a vector of arabis mosaic virus (Jha and Posnette, 1959, 1961; Harrison and Cadman, 1959) its ecology is of considerable importance to virologists, nematologists and the horticultural and agricultural industries they serve. It is now known that this nematode is widespread in England and Wales, although, despite much random sampling of soil for other nematodes, it remained undetected until 1958 (Goodey, Peacock and Pitcher, 1960). Preliminary surveys suggested that the distribution of the nematode was patchy and coincided remarkably closely with that of arabis mosaic infection in the crop plants studied. This paper reports on more detailed surveys carried out to investigate the natural distribution of *X. diversicaudatum* and its relation to that of the virus in land adjacent to two outbreaks of mosaic disease in strawberry.

METHODS

Soil samples, collected mainly during the spring and summer of 1960, consisted of up to 25 sub-samples per site, which were bulked and mixed before use. Whenever possible samples were taken with a 1-inch diameter corer of the cheese-sampling type to a depth of 9-inches; elsewhere a column of about 1-inch square section was cut from a slice taken with a spade to a similar depth. When the texture of the soil allowed, samples of 200 ml were passed through a standard Seinhorst elutriator (Seinhorst, 1956). Difficult soils were examined by the Baermann funnel method, 100 ml samples being placed on a milk filter held on a wire screen resting in a 6-inch diameter funnel filled with tap water. With the latter method nematodes were counted after 72 hours, the yield approximating to 75 per cent of that obtained from the elutriator for an equivalent volume of soil. Both techniques yielded a high proportion of larvae, but no distinction is made between these and adults in the results.

SITES EXAMINED

Sutton Valence, Kent. The nematode was found here, from 1958 onwards, in densities of up to 150 per 200 ml around arabis mosaic-infected strawberries growing in a garden plot made on a recently ploughed pasture (O.S.207), a silty loam over Weald clay. Further sampling revealed their presence around the roots of cultivated roses, *Astilbe* sp., *Cotoneaster* sp., raspberries, some healthy strawberries and in several turf areas. The survey then extended more or less radially from the garden, including pasture fields, plum, cherry and apple orchards, the intervening hedges and two oak-ash spinneys (Fig. 1). The principal components of the hedgerows were hawthorn *(Crataegus oxyacanthoides* Thuill.), blackthorn *(Prunus spinosa* L.), bramble *(Rubus fruticosus* L.), wild rose *(Rosa canina* L.), and maple *(Acer campestre* L.) together with a variety of herbaceous plants, mainly grasses.

Elham, Kent. The nematode was found here, in September 1959, around arabis mosaic-infected strawberries growing in an elite-stock bed (area O) established in 1958 in a corner of an old pasture on a chalk hillside. The survey covered this area and the remainder of the grassed field and the sur-

* Colombo Plan Student now at Extension Training Centre, Patna 1, Bihar, India.

rounding hedges and woodland, which contained a very varied flora, including hawthorn, blackthorn, bramble, rose, ash (*Fraxinus excelsior* L.), elder (*Sambucus nigra* L.), elm (*Elmus procera* Salisb.), hazel (*Corylus avellana* L.) and a variety of herbaceous plants, mainly nettle (*Urtica dioica* L.) and grasses (Fig. 1).

RESULTS

The maps in Fig. 1 show the general pattern of the distribution of the nematode. In Site 1 it was absent, or nearly so, in the pastures (O.S. 196, 201, 210, 216 and 217) and young grassed orchards (O.S. 204, 205 and 209). In contrast the nematode was readily found in all the intervening hedges sampled, sometimes in quite high densities. As rose is a known host plant (Schindler, 1957) several hedges were sampled differentially, sites under wild rose bushes being compared with others with a mixed flora without roses. In some cases the density in the rose sites was markedly higher than that in the corresponding non-rose sites, although in others this was not so. The mean numbers of *X. diversicaudatum* per 200 ml soil taken in hedges at Site 1 were:

Hedge No.	*Rose soil*	*Non-rose soil*
205/209	4	1
205/210	50	6
204/217	10	0·4
210/216	8	9
216/217	15	24

Two spinneys, one (adjoining O.S. 210) mainly of ash, with an undergrowth of hawthorn, bramble and grass, and the other very mixed, mainly of oak (*Quercus robur* L.), hazel and hawthorn with an undergrowth of rose, bramble, blackthorn and herbaceous plants, yielded the nematode in small numbers. Both spinneys were known to have been undisturbed for 150 years and probably for much longer. At Site 2 the nematode was numerous around volunteer strawberry plants on the site of the grubbed elite stock bed, but was scarce or absent in samples taken at random in the seven areas into which the rest of the field was sub-divided. Samples taken in areas predominantly of turf, wild white clover (*Trifolium repens* L.) or nettles yielded no indication of a host plant preference. The nematode was readily found, however, in hedgerows or woodland on three sides of the field.

TESTS FOR VIRUS INFECTIVITY

Infectivity tests for the presence of arabis mosaic virus in field soils were carried out by growing susceptible strawberry plants (var. Cambridge Favourite) or *Petunia* and turnip seedlings in 4-inch pots. The seedlings were tested after 6 weeks and the strawberry plants after 8–12 months by macerating their roots in phosphate buffer (pH 8·5 approx.) and using the sap to inoculate test plants of *Petunia* and *Chenopodium amaranticolor*. Arabis mosaic virus caused systemic symptoms in both these hosts and was identified serologically (Cadman, 1960).

Tests with soil collected at two depths near the roots of infected strawberry plants at Site 1 showed the presence of infective nematodes in all samples, even when the surface soil was extremely dry in September 1959 (Table I). In contrast, soil with dagger nematodes collected from around cultivated rose was not infective, neither were the tested samples from hedgerows.

At Site 2, arabis mosaic virus was recovered from soil around diseased strawberry plants and from the adjoining woodland and hedge, but not from other nematode-infested parts of the field, nor from the other infested hedgerow (Table 1).

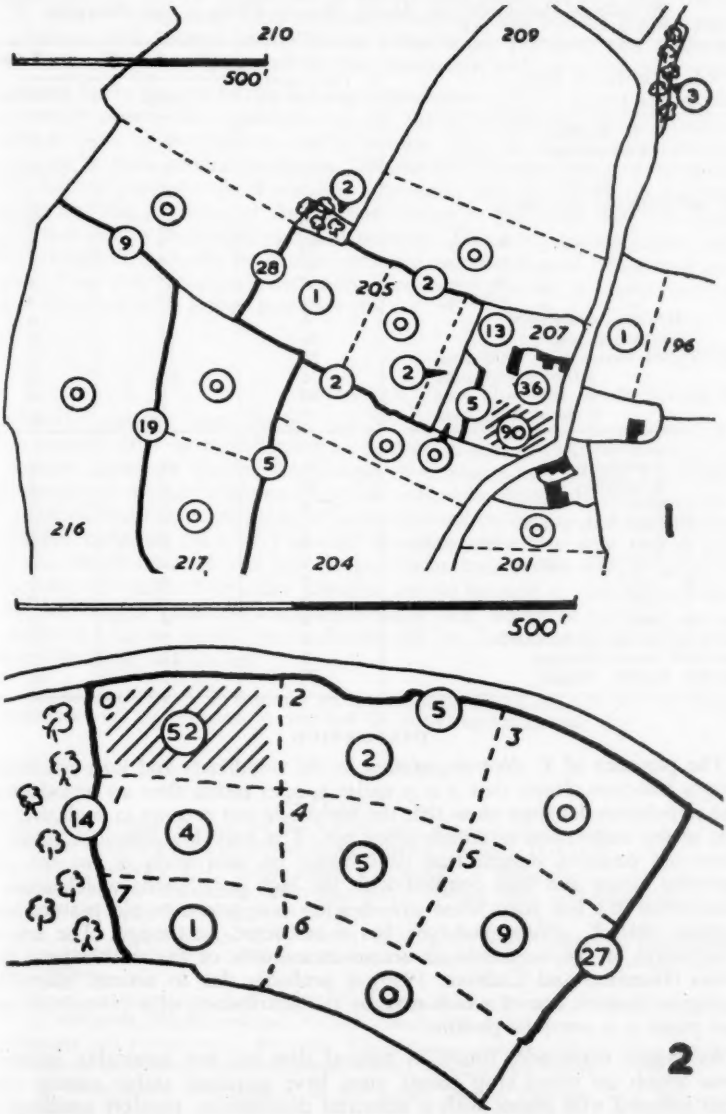

Fig. 1. Sites 1 (above) and 2 (below) showing (encircled) the mean numbers of *X. diversicaudatum* recovered per 200 ml of soil. Hedges sampled are indicated by thick lines and the diseased strawberry areas by cross hatching. O.S. numbers (site 1) and divisions of the field (site 2) are shown by figures in italics. The scale for each site is given *above* the figure.

TABLE 1

Results of Pot Tests for Arabis Mosaic Virus in Soil Samples

Source of soil	*X. diversicaudatum* per 200 ml	Number of pots of soil	
		Tested	Infective
E. Malling Control soil	0	10	0
Site 1			
OS 207 Infected strawberry	51	5	5
" "	77	10	9
" " 0– 9 in. ...	83	5	5
" " 9–18 in. ...	46	5	5
" " 0– 9 in. ...	150	5	5
" " 9–18 in. ...	51	5	5
Healthy strawberry	6	5	0
Cultivated rose	36	15	0
OS 205/210 Hedge with wild rose ...	50	18	0
without wild rose ...	6	18	0
OS 204/217 Hedge with wild rose ...	10	2	0
without wild rose ...	0·4	4	0
Site 2			
Area. 0. Strawberry	50	12	3
Strawberry	52	5	2
1. Turf	4	4	0
1. Turf with weeds	0	3	0
2. Turf with wild white clover	0	4	0
3. Turf with nettles	0	4	0
4. Turf	5	4	0
5. Turf	9	4	0
6. Turf	0	4	0
Western border (woodland)	14	9	3
Northern border (hedge)	5	10	1
Eastern border (hedge)	27	10	0

DISCUSSION

The presence of *X. diversicaudatum* in old woodlands and long-established hedges indicates clearly that it is a native species rather than an introduction. The population densities show that the nematode can prosper in such sites but that under cultivation it usually does not. The very low densities found in many old pastures suggest that disturbance by cultivation is not the only deterrent factor and this, coupled with the high populations which seem to have arisen in a few years when strawberries were grown on old pasture land, suggests that *X. diversicaudatum* has a restricted host-range. The uneven distribution of the nematode in near-monocultures of favourable crop host plants (Harrison and Cadman, 1959) is probably due to several interacting ecological factors, one of which may be the distribution of a favourable wild host plant in a previous pasture.

As dagger nematodes found in natural sites are not invariably infective, those which do infect crop plants must have acquired arabis mosaic virus from infected wild plants with a scattered distribution, possibly seedlings of common annual weeds (Lister, 1960a). Of the perennial wild plants known to be susceptible to the virus (Cadman, 1960), elderberry did not occur in the hedges at Site 1, but brambles were abundant. The nematode counts given in Table 1 suggest that wild rose is a host favourable to the nematode, but attempts to transmit arabis mosaic virus from these rose bushes have failed, although the virus can be readily transmitted to herbaceous test plants from

other Rosaceous hosts. The occurrence of non-infective dagger nematodes is in agreement with the experience of Hewitt and Raski *(in lit.)*, who found that *X. index* Thorne and Allen collected from figs and healthy grapes were not infective with fanleaf virus. On the other hand, Harrison and Cadman (1959) found *X. diversicaudatum* only in association with virus-infective soils.

It seems likely that in future *X. diversicaudatum* will be found to be still more widely distributed, especially on the site of woodlands or hedgerows. In certain parts of the country arabis mosaic virus is already of economic importance to fruit producers (Lister, 1960b) but the problem is even more serious for the propagators of certified strawberry and raspberry stocks. Soil sampling for the presence of dagger nematodes in proposed sites for such propagation is now becoming standard practice. This investigation shows that particular attention should be paid to grubbed woodlands and hedgerows and suggests that wide headlands left unplanted may provide a useful barrier against infection with arabis mosaic virus.

SUMMARY

Systematic surveys were carried out in the vicinity of two outbreaks of arabis mosaic virus in strawberries to determine the natural distribution of the dagger nematode vector, *Xiphinema diversicaudatum* (Micol.) Thorne. In pastures and orchards surrounding the outbreaks dagger nematodes were rare or absent but they were plentiful in the intervening hedges and woodlands. Nematodes collected from soil around the diseased strawberry plants and from one woodland area and one hedge transmitted arabis mosaic virus to herbaceous test plants. Particular attention should be paid to hedgerows when surveys for dagger nematodes are undertaken and, wherever possible, crops susceptible to arabus mosaic virus should not be planted close to hedges or on newly-grubbed woodlands.

We wish to thank Dr. A. F. Posnette for much helpful advice and Mr. J. J. M. Flegg for assistance with the nematode survey and for preparing the figures.

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EFFECTS OF STREAK VIRUS ON THE GROWTH OF COCKSFOOT

by P. L. CATHERALL *and* ELLIS GRIFFITHS

Department of Agricultural Botany, University College of Wales, Aberystwyth

COCKSFOOT streak virus, first described in 1952 (Storey, 1952; Smith, 1952) is now known to be widespread in Britain. The virus usually produces a very conspicuous longitudinal streaking of dark and light green or yellow, most easily discernible in the summer and early autumn. Sometimes, however, the mottle is inconspicuous. Infected plants are often paler in colour, usually developing a "floppy" appearance and may be picked out relatively easily from some distance away. Although by no means characteristic, a marked dwarfing frequently occurs in infected plants.

The virus has been aphid transmitted by *Myzus persicae* and *Macrosiphum euphorbiae* (Smith, 1952), by *Hyalopteroides humilis* and other species (Mulligan, 1961), but success has been variable. The virus can also be manually transmitted again only with difficulty.

Little is known of the effects of cocksfoot streak on the productivity of the host. Under pot conditions, Smith (1952) reported that the virus did not produce a marked deterioration of the plant. A specimen infected with streak, kindly supplied by Dr. K. M. Smith two years ago, and since broken down into several units, although always possessing a marked mottle, still continues to survive both in the greenhouse and in the field without apparent loss of vigour. More recently, however, the virus appears to have caused severe damage to cocksfoot varieties in trials at the Grassland Research Institute, Hurley. Specimens obtained from both Hurley and the Welsh Plant Breeding Station and grown at Aberystwyth, although conforming in symptom picture to the plant supplied by Dr. Smith, suffered to varying degrees both in the greenhouse and in the field. The most striking effects were shown by infected plants collected at Hurley in April, 1959. Throughout the summer these plants made little or no progress and out of nine plants kept in pots in the greenhouse six were dead before the following spring, while the other three, believed to be only partly infected in April, lost their infected tillers, the remaining tillers continuing to survive without symptoms. Consequently it can be concluded that a wide range of variation exists in the host-virus interaction, actual mortality emphasising the need for a study of the agronomic importance of this disease.

MATERIALS AND METHODS

All material used in the experiments described here was obtained from the Welsh Plant Breeding Station, Aberystwyth, where both healthy and infected plants of the same genotype were available. Mr. T. E. Mulligan at Rothamsted who kindly examined the material was able to show that the virus was transmitted by aphides but not by mites, thus proving that the plants were not infected with rye-grass mosaic to which cocksfoot has also been shown to be susceptible (Mulligan, 1960).

Healthy and infected plants of identical genotype were broken down into single tillers during the winter of 1958-59 and planted in boxes kept in the greenhouse under supplementary light. After establishment, an equal number of healthy and infected individuals, now in the three to four tiller stage, were

selected for uniformity of growth. These were transferred during late April 1959 to 7-inch pots containing sterilized soil low in minerals. The pots were kept outside for the duration of the experiment. Eight fertilizer treatments involving all combinations of nitrogen, phosphate and potash were applied to both groups of plants, each treatment being repeated three times. The fertilizers used were sulphate of ammonia, superphosphate and muriate of potash, each applied in solution at the rate of 1 cwt per acre. All plants were then cut to a standard height of 3 inches and the pots arranged as a randomized block. These cuttings were continued each month throughout the season and the fresh and dry weight yields determined for each plant. At the time of cutting the tiller number of each plant was also recorded and the appropriate fertilizer treatment repeated.

As cocksfoot is a herbage plant normally grown in a sward it is important to determine the effects of virus infection on plants under these conditions. Small experimental swards were established in the following manner. Large wooden boxes (20 in. × 15 in.) with extensively perforated bases were filled with sterilized soil to which Fisons 35 compound fertilizer was added at a rate of 1 oz. per box. The selected plants were then broken down into single tillers, and these transplanted at random into the boxes at about two-inch spacing, nine by seven to the box. Large numbers of plants were required for this experiment and as it was not possible to obtain sufficient healthy and infected plants of the same genotype the material used consisted of eighteen randomly selected healthy plants and eighteen randomly selected plants of the same strain (W.P.B.S. No. B.90). Each box was mapped so that the parental identity of any tiller could be determined. In this way " swards " of all-healthy, all-infected and a mixture of equal numbers of healthy and infected plants arranged alternately were prepared and placed in a heated greenhouse until the tillers were well established.

The mortality in the establishing tillers was 5 per cent in the case of the healthy and 23 per cent in those infected with virus. Consequently before the " swards " were transferred to their permanent site they were balanced by adding tillers of the same parentage, established under similar conditions, or, where there was insufficient identical material, by the removal of corresponding plants in the appropriate boxes. On completion, all " swards " contained a full complement of 54 established plants. The " swards " were then transferred out of doors and arranged in a randomized block, the boxes being embedded almost to the rim in soil recently limed and fertilized to facilitate the full development of the plant root systems through the perforated base of each box. The swards were cut to a standard height of 2½ inches. The three types of sward (healthy, 50 per cent infected and 100 per cent infected) were each repeated six times. To these two cutting treatments were applied. Each month one half of the swards were cut to standard height and the fresh and dry weights of the cut herbage recorded. The other half of the swards were left uncut until the end of the season when the total fresh and dry weight yields of the herbage were obtained in one cut. In October, 1959, the tiller number of each plant was recorded together with the percentage mortality of established plants. A further check on mortality was made in March, 1960, to ascertain how well infected plants overwintered.

RESULTS WITH SINGLE PLANTS

Comparisons were made between infected and healthy plants under different fertilizer treatments in regard to tillering, dry-matter yield and dry-matter yield per tiller. Although the effects of all combinations of N, P and K

on these characteristics were studied, only main effects are here considered. Significant interactions were rarely encountered and appeared to be of only minor importance.

Tillering. One of the most striking effects of virus infection was a marked reduction in tillering; on average infected plants produced only about 60 per cent of the number of tillers produced by healthy plants (Fig. 1). All fertilizer treatments tended to increase tillering in both infected and healthy plants but statistical analysis of the data revealed that the two types of plant did not respond equally to all the treatments. Thus nitrogen and potash produced significantly greater responses in healthy plants whereas phosphate produced an equal response in both groups.

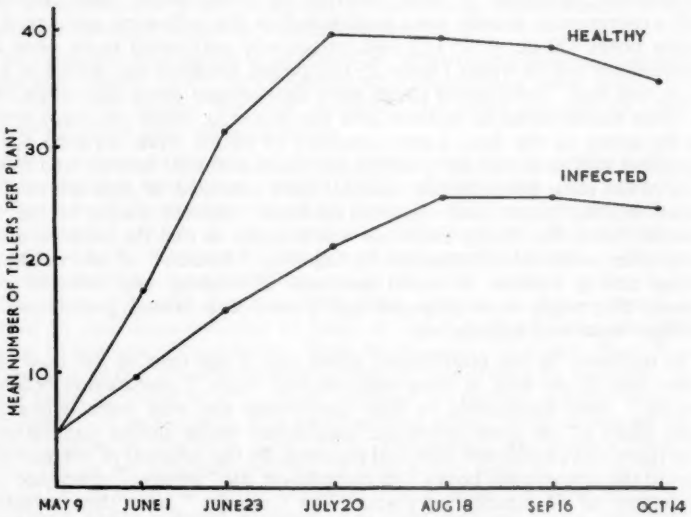

Fig. 1. Tiller production in healthy and virus-infected cocksfoot plants.

Dry-matter Yield. Despite the large differences in tiller production the dry-matter yield of infected plants was on average reduced only by about 10 per cent. The one fertilizer which significantly increased dry-matter yields was nitrogen and again healthy plants responded to a greater extent than infected ones. Although no significant effects could be attributed to either phosphate or potash there was a slight indication that infected plants responded more to potash than did healthy ones.

Dry-matter Yield per Tiller. Tillers of infected plants were observed to be generally larger than normal and the dry-matter per tiller was found to be almost 30 per cent higher. Application of nitrogen significantly increased the dry weight of tillers but there was no evidence of any differential effect on healthy and infected plants. No effects of phosphate on this point could be detected; potash on the other hand produced an interesting differential effect. The overall effect of potash was not significant but there was an indication of a difference in its effect on healthy and diseased plants. This effect just

failed to reach significance at the 5 per cent level when the data for the whole season were analysed jointly but analysis of individual cuts showed highly significant effects for the months of August and September. From Table 1 it can be seen that the difference between healthy and infected plants in the absence of potash was 20 per cent but that in its presence this increased to over 50 per cent.

TABLE 1

Effect of Potash on Dry-Matter Yield per Tiller (mg.) in August and September, 1959

	Healthy	Infected	Difference *per cent*
With potash	58·9	129·0	54·0
Without potash ...	77·6	97·2	20·2

RESULTS WITH "SWARDS"

Dry-matter Yield. The total dry-matter yields from the three types of sward (all-healthy, 50 per cent infected, and all-infected) are shown in Table 2. The all-healthy swards outyielded the all-infected by over 30 per cent but surprisingly the total season's production for both was unaffected by cutting treatment. This was not so in the mixed swards. Here, with monthly cutting, the yield was close to that of the healthy swards, whereas when cut only once the yield approached more closely that of the all-infected swards.

TABLE 2

Total Yield of Dry-Matter (g.) from "Swards"

	All-healthy	50 *per cent* infected	All-infected
Cut monthly	187·0	169·1	124·3
Cut once	194·4	137·1	127·1
Total	381·4	306·2	251·4

In September, when all the swards were cut, the herbage from healthy and infected plants of mixed swards was harvested separately so that the contribution of each component could be assessed. The mean values are shown in Table 3. It can be seen that with monthly cutting the growth of the healthy plants was greater than in the all-healthy while that of infected plants was slightly reduced. On the other hand with a single cut healthy plants yielded slightly less than those in all-healthy swards whilst the yield from infected plants was substantially less than from those in all-infected swards.

TABLE 3

Mean Dry-Matter Yield per Plant (mg.) from "Swards" in September, 1959

	All-healthy	50 *per cent* infected		All infected
		Healthy plants	Infected plants	
Cut monthly ...	158·5	227·5	78·5	88·8
Cut once ...	849·4	817·9	485·5	605·2

Death of Infected Plants. Virus infection, in addition to decreasing dry-matter yields, has also been found to increase plant mortality. This amongst single plants in pots whether healthy or infected was nil but under sward conditions death of plants occurred in both groups. Inspection of the swards in September 1959 showed the mean loss amongst healthy plants to be just under 2 per cent; amongst virus infected plants it was 4 per cent. By the end of March 1960 these values had increased to 4 per cent and 14 per cent for healthy and infected plants respectively. The mean values for the various treatments are shown in Table 4 from which it can be seen that in general the greatest losses, in both healthy and infected plants, occurred in swards cut once.

TABLE 4

Mortality (per cent) of Healthy and Infected Plants in "Swards"

	All-healthy		50 *per cent* infected				All-infected	
			healthy plants		Infected plants			
	Sept. '59	Mar. '60	Sept. '59	Mar. '60	Sept. '59	Mar. '60	Sept. '59	Mar. '60
Cut monthly	0·0	1·9	1·2	3·7	2·5	13·6	1·2	11·7
Cut once ...	2·5	4·3	3·7	6·2	6·2	12·3	5·6	21·0

DISCUSSION

In considering the effects of cocksfoot streak it is important to appreciate that the particular host-virus combinations so far examined do not represent the most severe form of the disease. Material obtained from the Grassland Research Institute, Hurley, shows much more extreme effects and preliminary studies here indicate that a different strain of the virus may be involved.

Studies on single plants have shown that the symptoms and effects of the virus are least under conditions of low fertility, particularly low nitrogen, a finding which is in accord with results obtained by workers on other virus diseases. For example, increase in severity of symptoms has been observed in cauliflower mosaic (Broadbent and Tinsley, 1953) and barley leaf stripe in spring wheat (Army and McNeal, 1958); for the same disease McNeal, Berg, Afanasiev and Army (1958) also found the difference in productivity between healthy and diseased plants increased with application of nitrogen. The effect of potash in promoting disease resistance is well known (Lawson and Cook, 1954) but its effect on established infections has received less attention. In the case of cocksfoot streak the main effect of potash is to increase, at certain times of the year, the dry matter yield per tiller, thus to some extent offsetting the deleterious effects of reduced tiller production.

The study of single infected plants, though it certainly provides useful information about the nature and possible extent of loss, does not afford a reliable means of appraising losses which may occur when the same plants are grown as a group. The reduced growth of infected individuals will always be compensated to some extend by increased growth of adjoining healthy ones. Even in crops such as sugar beet where individual plants are well spaced the compensation effect may be substantial (Jones, Dunning and Humphries, 1955); in a grassland sward where severe inter-plant competition occurs it will be at a maximum. Further, grassland is subject to far greater variation in management than any other crop and this further complicates the evaluation of the significance of disease in pasture plants. In our studies on artificial

swards two contrasting managements have been examined and it is evident that their influence on the behaviour of infected plants is considerable. In the mixed swards kept closely cut the extra growth of health plants was almost sufficient to compensate entirely for the reduced growth of infected ones. With only one cut, however, there was no evidence of any compensation. Evidently the practical significance of the disease is likely to be least in well grazed swards and greatest in fields put up for hay.

In a perennial crop survival of virus infected plants may lead to progressive deterioration. Under the highly competitive conditions in a grassland sward, however, weak plants naturally tend to be eliminated and replaced by more vigorous ones. Thus it would be expected that virus infected cocksfoot plants would tend to disappear from a sward. Such a view is supported by the information at present available. Even in the winter of 1959–60 when no very cold periods occurred at Aberystwyth and when no mortality was recorded in spaced single plants (Beddows, *in lit.)* losses of infected plants in swards amounted to about 15 per cent. Under more rigorous conditions much higher losses would be expected. In swards substantially infected by streak this would lead to patchy growth in the following spring and might well lead to reduced productivity at this time.

We are indebted to Mr. A. R. Beddows, Welsh Plant Breeding Station, Aberystwyth, for providing the healthy and infected cocksfoot plants used in these experiments and to Mr. T. E. Mulligan, Rothamsted Experimental Station (now at the Agricultural Institute, Dublin) for confirming the identity of the virus in our infected material.

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TRIALS OF FLUORESCENT TRACERS FOR INSECTICIDES IN SOIL

by L. N. STANILAND
National Agricultural Advisory Service, Bristol

PREVIOUS papers (Staniland, 1959 (a and b) and 1960) give information on a range of fluorescent tracers and their use in the field for the study of spray and dust deposits and of the efficiency of various cultivation methods for the incorporation of insecticide dusts and fluids into soil. One of these papers sets out the sources of supply of tracers and gives details of their formulations (Staniland, 1959b). In this present paper the methods used in soil tracing are considered and some of the results obtained are discussed.

The principle of tracing with fluorescent materials is that these, in powder or liquid form, are used to simulate insecticide sprays and dusts, or are added to recognised insecticidal formulations. Viewed under ultra-violet light in a darkened room, the distribution of insecticide is readily seen on plants or in the soil. The source of ultra-violet light may be an ordinary 150-200 watt lamp screened by Wood's glass; or a mercury discharge lamp with a protective bulb of Wood's glass. An ordinary electric lamp with Wood's glass is suitable for the brighter tracers and can be switched on or off as required. The mercury discharge lamp must be used with a "low power-factor ballast," and if switched off cannot be switched on again until the lamp has cooled; the discharge lamp is more powerful and is better suited for use with the less brilliant tracers; it does not demand such a dark room for satisfactory use.

TRACERS USED IN SOIL WORK

Fluorescent powders may be used in dusts, or in liquid suspension, in amounts sufficient for tracing purposes, or in quantities equal to the toxic chemical present. Test materials should be sub-sampled and examined under ultra-violet light with a microscope to ensure that mixing is thorough. All the following powders are relatively stable and may be used in experiments extending over some months in the open:

Phosphorescent Powder, Green: This fluoresces a brilliant light green, but is also luminescent and continues to glow in the dark for some time after activation by ultra-violet or ordinary white light. If any particles suspected as being naturally fluorescent are seen in soil, contamination can be tested by intercepting the ultra-violet light falling on the soil; added luminescent material continues to glow, but purely fluorescent material ceases to be visible. Naturally fluorescent materials are present in soil, but in my experience always fluoresce in blue or violet shades. Thus tracers fluorescing in yellow, red or green are preferable, particularly if luminescent as well.

Anthracene, Technical Grade: Used in finely powdered form, fluoresces a brilliant bluish-green.

Saturn Yellow and Neon Red: Very finely divided and valuable in dusts or as a suspension in liquid insecticides. Fluoresce a brilliant sulphur yellow and orange-red respectively.

There are a number of oil soluble fluorescent dyes on the market, of which "oil colour G" is particularly suited to soil work. This is soluble in various organic liquids, including ethanol and solvent naphtha. It is used 0·2 per cent by weight and is only light-fast for about five days in the open.

TRACING INSECTICIDES IN SOIL

Dusts containing luminescent or fluorescent powders were mixed with inert carriers, such as kaolin, to simulate various insecticidal dusts, or the amount of tracer equivalent to the percentage of toxic chemical was added to a commercial dust. The traced dust was then applied to the soil surface and worked in by various cultivation methods, the efficiency of which it was desired to assess. Random areas of 5 sq. yd of treated soil were marked off; from these areas 50 soil samples were then taken to the desired depth with a cheese sampler. Each sample, as it was taken, was divided into sections according to the various depth levels to be studied. All the samples from each depth were bulked. These bulk samples were then air-dried and passed through a 2 mm sieve to break them into a fine powder and remove stones and plant debris; each sample was then very thoroughly mixed. An estimation of the numbers of fluorescent particles present in the various samples was made as follows:

About 10 g of the dried soil was taken from the samples at random and spread out in a petri-dish to a depth of about $\frac{1}{4}$ inch. The soil surface was then examined under ultra-violet light with a $\times 25$ binocular microscope, a pair of condensers being used to concentrate the light. The area of the microscope field for this power was almost exactly $\frac{1}{4}$ inch square. Estimates of the number of fluorescent particles in cubic units of soil can be made if required, but for working out the approximate percentage of active chemical present in the various depths of soil it is not necessary to know the area of the microscopic field so long as the same field is used throughout. When very fine powders are used it may be found necessary to use a higher power with a consequently smaller field. A magnification of $\times 100$ gives a field approximately equal to a 1/16th inch square. Aggregation of the fluorescent particles occurs equally in all samples and does not materially affect the results. The results are not assays giving information on the amount of toxic chemical present in any depth level, but are close approximate estimates of the percentage of toxic chemical present at various levels. The results also apply only to insoluble materials.

The number of fluorescent particles (or small aggregates) present in the microscopic field is counted. The dish is then moved at random to observe another field. About 20-30 fields may be counted in each petri-dish sample, a total of 100 random counts being made for each depth sample. The numbers of particles counted in two test series of 100 microscopic fields were as follows:

	No. of microscopic fields with mean count per field						Mean count per field
	0	1	2	3	4	5	
Test 1	47	34	9	5	0	5	0·92
Test 2	20	45	21	11	3	0	1·32

It will not often be found necessary to count more than 50 particles in any one field—frequently much less—except in soil very close to the surface, where the work has shown there is usually a high concentration of dust. Counting particles in such samples is tedious and good estimations may be made by comparing the concentration of fluorescent particles seen in the microscope field with a known standard. A series of circular cards of stiff dark paper are cut out to approximate the field in apparent size when laid on the bench beside the microscope. A fluorescent powder, such as Saturn Yellow, is mixed with a thin celluloid varnish and applied as small dots to the cards with a pen or fine brush. The dots are evenly spaced by eye over the circles; cards with 50,

75, 100 and 200 dots should be made, others being prepared in steps of 100 up to 1,000. The cards are lit by the ultra-violet lamp and the dots glow brightly against the dark background.

Some difficulty may be experienced in making notes in semi-darkness. Two methods are helpful: make up a paint with celluloid varnish and the green phosphorescent powder and coat a piece of plywood with it; when dry bind on a protective piece of glass. The board will glow strongly in the ultra-violet light. Lay tracing paper over the board and writing is easy. Alternatively, brush over sheets of paper with a fluorescent paper whitening dye (such as Fluolite C), or a bright yellow water soluble fluorescent dye. When dry the sheets glow in ultra-violet light and can be written on. Some papers, as sold, already contain such optical whitening dyes.

THE DISTRIBUTION OF INSECTICIDE DUSTS IN SOILS

Laboratory tests were made with soil/tracer mixtures to provide estimates of the amount of toxic chemical present after dusts were incorporated thoroughly into fine dry soils. The soil was measured in a 6 cubic inch box, calibrated in depth. A dust containing 1¼ per cent of green luminscent powder (simulating active chemicals) was then accurately weighed out to give several rates of application.

At the rate of 800 lb per acre (equivalent to 10 lb a.i. per acre), mixed 6 inches deep, the mean number of fluorescent particles per ¼ inch square was 0·72. When the dust was mixed at the rate of 1,600 lb per acre the number of active particles was 1·18. This shows that the method is sufficiently accurate for the general study of such problems. Supposing that, in field practice, dusts do not become thoroughly mixed to a depth of 6 inches, but only to 2 inches, the 800 lb per acre rate then gives a particle count of 2·03, or approximately three times the figure when the dust was mixed 6 inches deep.

Assuming that the active particles are distributed in depth as seen in one plane, it follows that a soil insect would find at the most about two particles per cubic ¼ inch when the 1¼ per cent dust is applied at 800 lb per acre and mixed 6 inches deep; this is about 128 particles per cubic inch. When, however, the same unit of dust is mixed only 2 inches deep there would be about eight particles per cubic ¼ inch, and over 500 per cubic inch. Whether a dust is mixed thoroughly to a depth of 6 inches, or only to 2 inches, might therefore have a profound effect on the control of a soil pest.

This matter was pursued further by taking as an example the application of a dust containing 3½ per cent of the luminous powder to simulate a 3½ per cent BHC dust. It was applied at the rate of 2 cwt per acre, and mixed to a depth of 2 inches, a likely depth to which it might be incorporated by harrowing. The number of active particles per ¼ inch square was 2·24, giving a figure of some 700 particles per cubic inch. BHC, used in this way, is effective against wireworm, and the particle concentration of 700 per cubic inch would seem to confirm this.

When a dust is mixed with a fine dry soil it can be evenly distributed; but when the soil is damp, with a definite crumb structure, the dust particles adhere to the surface of the crumbs and are not easily removed. These effects were examined and the results are set out in Table 1. The soil used was a light sandy loam, low in organic matter and very free of stones. The mean number of particles observed per ¼ inch square are those with which an insect might be expected to make contact, it being assumed that particles locked within soil crumbs would not be effective. The dust used throughout contained 3½ per

cent of the luminescent powder and was applied at the rate of 2 cwt per acre, 2 inches deep.

TABLE 1

Distribution of Dust according to Soil Crumb Structure

State of soil and mixing of dust	Mean active particles per ¼ inch square	Remarks
Dust mixed with fine dry soil	2·24	Particles fairly evenly distributed throughout the soil.
As above, then dampened to an ½ inch crumb	0·79	Much active material locked up within the soil crumbs.
Untreated fine dry soil dampened to an ½ inch crumb and then mixed with dust ...	1·96	Active particles very unevenly distributed on the outsides of the crumbs. Some crumbs with heavy coating and others with very few particles adhering.
As above, after drying, sieving and mixing	2·11	Particles released from crumbs and again fairly evenly distributed throughout soil.
As above, reconstituted to ½ inch crumb after moistening	0·58	Many of the particles again locked up within the crumbs, but particles on the surface more evenly distributed than when untreated soil was moistened before mixing with tracer.

The results in Table 1 were sufficiently encouraging to warrant field tests of the efficiency with which hoeing and rotovation incorporate dusts into the surface soil. Many of the smaller growers hoe dusts in. To test the efficiency of this method dust was applied evenly to the surface of 4 yd by 2 ft plots at the required rate, and was then hoed in as thoroughly as possible by means of a three-tined hoe to a depth of 6 inches; rather more time being spent on the hoeing than would occur in practice. The soil was light, sandy, and dry, with many lumps and stones. Dusts of different concentrations and rates of application were used, the tracer again being the green luminescent powder. The results are shown in Table 2.

The percentage of active chemical in the various 1 inch depths is very similar, whether a dust containing 1¼ per cent a.i. is applied at 3 cwt per acre, or a 5 per cent dust at 2 cwt per acre. In both cases the bulk of the chemical remains in the top 1 inch of soil, less than 20 per cent in the 1-2 inch layer, about 5 per cent in the 2-3 inch layer, and below that only very small amounts. With the 5 per cent dust at 2 cwt per acre, 11·2 lb a.i. is applied per acre, but hoeing leaves 8·2 lb still in the top inch. The greater amount of active chemical applied where the 5 per cent dust at 2 cwt per acre was used, as compared with the 1¼ per cent dust at 3 cwt per acre, was reflected in the mean counts of fluorescent particles per ¼ inch square; the figures for the 0-1 inch layer were 4·77 and 3·17 respectively, and indeed the differences were appreciable to the eye.

A rotary hoe was used to incorporate a 1¼ per cent dust (at 3 cwt per acre) a full 6 inches deep in larger areas than were used in the hoeing experiments. The soil was also of a rather heavier type and more moist. A comparison was made between one and two rotovations. The results are shown in Table 2: one

rotovation is far more efficient than hand hoeing, only some 30 per cent of the active material remaining in the top 1 inch, and 8 per cent was to be found in the 4-5 inch layer. A second rotavation mixed the material more evenly at the various depths, though the 5-6 inch layer still contained little. These two rotovations suggest that the soil is not inverted during the progress.

TABLE 2

Estimates of Insecticide present at a range of Soil Depths after different Cultural Treatments

Treatment	Estimated Percentage of Insecticide, at inches from surface:					
	0–1	1–2	2–3	3–4	4–5	5–6
1¼ per cent dust at 3 cwt per acre hoed in ...	73	18	6	1	1	1
5 per cent dust at 5 cwt per acre hoed in ...	72	17	5	3	2	1
1¼ per cent dust at 3 cwt per acre rotovated once to 6 in.	34	23	22	11	8	2
1¼ per cent dust at 3 cwt per acre rotovated twice to 6 in.	27	15	25	17	13	3
Soil drench with 1 per cent traced oil emulsion, soil fine and dry ...	83	17	— traces only —			
Soil drench with 1 per cent traced oil emulsion, soil damp with fine crumb	60	12	7	7	7	7
Traced aldrin spray, followed by 3 tine-harrowings, cultivation, and planting of potatoes ...	— 62 —		— 21 —		— 17 —	
Traced aldrin spray, as above, but samples taken after two ridgings from level of new tubers upwards	— 22 —		— 27 —		— 51 —	

THE DISTRIBUTION OF LIQUID INSECTICIDES IN SOIL

Experiments were first made with an oil emulsion spray in the laboratory. Saturn Yellow powder tracer was ground with rape oil in the proportion of approximately 10 per cent by volume. The oil was then emulsified with oleic acid and caustic soda and used as a dilution of 1 per cent. Each droplet of oil in the emulsion contained fine particles of the tracer and thus marked the site of the oil droplets after the treated soil had dried. Dry and damp soils were placed in waxed paper cylinders 4 inches in diameter and 6 inches high, marked off by ring-lines into inch depths. The cylinders were stood in dishes full of soil. The traced emulsion was poured gently and evenly over the surface of the soils until run-through occurred. The cylinders of soil were then left until quite dry. Adhering soil, on which the cylinders had stood, was then pared level with the base of the cylinders, and, after cutting carefully round the depth lines in turn with scissors, each layer of soil was cut with a wire, slid off on to a flat surface, and examined. Preliminary tests, where the column of soil was sectioned vertically, showed that oil emulsion droplets were readily sieved out by soil and largely retained in the upper layers, with a very con-

centrated layer of the spray material lying on the soil surface. Further tests threw some light on the condition of the soil in relation to this fact.

With fine dry soil, lightly compacted, it was found that some 500 cc of the emulsion was required before run-through occurred, and the process was slow. The surface of the soil soon broke down to a fine mud so that parts of the soil surface became relatively impervious to water which lay in small pools; the overflow from these pools continued to penetrate. This phenomenon is known as " ponding " and usually occurred when about a third of the emulsion had been poured. The results are shown in Table 2: 83 per cent of the oil droplets were in the top inch, much on the surface, 17 per cent in the next inch, and below this only very small amounts were found.

With damp soil lightly compacted in a fine crumb state, less liquid was required before run-through took place; the whole process was much quicker and " ponding " did not occur until about three-quarters of the 350 cc required had been poured on. The results, in Table 2, show only 60 per cent in the top inch, 12 per cent in the next inch, and below that approximately 7 per cent in each 1-inch layer down to the bottom. Further experiments on similar soils were carried out using a commercial DDT emulsion traced with the oil soluble fluorescent dye G. This emulsion was finer than the rape oil emulsion. Since it contained no particulate tracer it was not possible to make counts, but it was readily observed that penetration into the damp soil was much better; with the fine dry soil the distribution was similar to that when rape oil emulsion was used.

Further tests were made in the field. A grower wished to know whether an aldrin spray applied at low volume to the surface of the soil, prior to the planting of the potatoes, would be satisfactorily distributed in the soil by harrowing and cultivating. The grower mixed seven pints of 15 per cent emulsion concentrate with 30 gallons of water; to this was added 1½ oz Saturn Yellow powder mixed to a thin cream with a little wetter (without which it is most difficult to wet). The spray was applied by a low volume sprayer with flat-jet nozzles, on May 13 at 30 gal/a. The soil was a light sandy loam, derived from Keuper sandstone, and was fine and dry. After spraying, the field was tine-harrowed three times, and cultivated once. Potatoes were then planted on the flat by drawing out shallow furrows, closing them in and then giving a final light harrowing. On May 28, 20 random samples 1½ inch diameter and 6 inch deep were taken. These were sub-divided and bulked into 2 inch depths and examined as usual. The results are shown in Table 2: nearly two-thirds of the material was dispersed in the top 2 inches and 17 per cent in the 4-6 inch layer. During the second and last weeks of June the potatoes were ridged, first lightly and then higher. In August a composite sample of about 5 lb of soil was taken by hand from 20 sites in the ridges. One bulk sample was from just above the tubers, another from just below. After drying, mixing and sieving these soils were examined. While both had plenty of the spray material present, it was at once noted that there was obviously more material below the potatoes than was present just above them and counts made in the usual way showed that the difference was approximately 14 per cent.

On September 30, 20 further samples 1½ inches diameter and 6 inches deep were taken, reaching to just below the level of the tubers, and the results are shown in Table 2: the order of concentration in the two-inch layers is now reversed as compared with that obtaining in the samples taken soon after planting. This confirmed the fact that most of the chemical was to be found just above and below the tubers, and it is of interest that this soil depth, which is clearly most important from the point of view of wireworm control, contains

half the insecticide present in the total surface to 6 inch depth. It would appear that it is the ridging up process which has reversed the order of concentration of the soil layers.

SUMMARY

The use of fluorescent materials for tracing insecticides in soils is described, together with methods for sampling and examination of soils.

The mixing of insecticide dusts with dry, finely sieved soil in the laboratory has shown that the concentrations of a toxic chemical available to a soil insect will vary greatly (apart from such factors as volatility) according to the depth of soil with which it has been thoroughly mixed. The state of the soil also affects the distribution of the material; if the soil is dry it can be evenly distributed, though frequently it is not. When the soil is damp and has a fine crumb structure, a dust becomes distributed on the outside of the individual crumbs. Subsequent mixing of the soil distributes the contaminated crumbs throughout the mass, but this does not result in transfer of the dust to other crumbs; the result is uneven distribution. If a soil is fine and dry and a dust has been well mixed with it and the soil is then moistened by rain and cultivated, much of the insecticide can be locked up within the soil crumbs. In this position is may not be active against soil insects. If, later, this soil dries out and is cultivated to a fine state again, the insecticide is released and becomes active again. This suggests that the condition of the soil when a dust is applied, and subsequent dampings, dryings-out and cultivations, will be likely to bring about fluctuations in the effectiveness of the treatments.

Hoeing to a depth of 6 inches leaves about three-quarters of the material in the top 1 inch. Rotovation is more efficient than hoeing and two rotovations are better than one.

When large amounts of a liquid emulsion insecticide are applied as a drench, fine dry soils require more liquid to saturate them than damp soils; a high concentration of active material remains on the surface, most of it in the top 1 inch.

In damp soils, with a fine crumb structure, ponding tends to occur later; less of the material remains in the top 1-2 inches, and far more in the lower levels than with fine dry soils. The finer the emulsion the better the penetration.

When an emulsion insecticide is applied at low volume and is worked into the soil by various cultivations, over half the material remains in the top 2 inches. If potatoes are planted on the flat and subsequently ridged twice, the order of concentration in the soil layers is reversed. Then about half the insecticide material is found in the layer of soil in which the new tubers are produced.

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